The development of ^{11}C-sorafenib, a potentially new PET-tracer

Berend van der Wildt
Utrecht University- Drug Innovation
Internship at the RadioNuclideCentre
Department of Nuclear Medicine & PET Research, VU University Medical Centre
January 2010 – October 2010

Supervision of Dr. Alex Poot, Dr. Bert Windhorst and Prof. Dr. Guus van Dongen

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List of abbreviations

AUC	area under curve
Boc	*tert*-butyloxycarbonyl
c-Kit	cytokine receptor on hematopoietic stem cell surface
CT	computed tomography
DCM	dichloromethane
DMF	dimethylformamide
DPPE	1,2-bis(diphenylphosphino)ethane
EGFR	epidermal growth factor receptor
ERK	extracellular-signal-regulated kinase
FDA	Food and Drug Administration
FDG	fluoro-deoxy-glucose
Flt-3	FMS-like tyrosine kinase 3
HPLC	high performance liquid chromatography
IC_{50}	concentration of 50 % inhibition
MAPK	mitogen activated protein kinase
MRI	magnetic resonance imaging
MS	mass spectrometrie
NMR	nuclear magnetic resonance
PDGFR	plateled-derived growth factor receptor
PET	positron emission tomography
TBAH	tetrabutylammonium hydroxide
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Tf	triflate
UV	ultraviolet
VEGFR	vascular endothelial growth factor receptor

Summary

The multi-target kinase inhibitor sorafenib is clinically used for the treatment of various tumours. In this thesis sorafenib is labelled with carbon-11, a PET-isotope, for the prediction of response to therapy before the start of treatment. Three strategies are explored for the introduction of a radiolabel at the urea or the methylamide functionality of sorafenib. Both variants of [^{11}C]sorafenib are synthesized and formulated successfully for evaluation in an *in vivo* model of tumour bearing mice by dynamic PET-scanning.

Introduction:

Kinases and kinase inhibitors

Kinases include a large class of enzymes that are responsible for the reversible phosphorylation of proteins at their serine, threonine or tyrosine residues. By this phosphorylation the kinases can activate their substrate-proteins and thereby regulate diverse functions in the cell, such as metabolism, transcription, cell cycle progression, cytoskeletal rearrangement and cell movement, apoptosis, and differentiation. To date, over 500 kinases are discovered and indentified in man[1]. Figure 1 shows the classification by substrate of these kinases in groups, families and subfamilies in a dendrogram. The 7 main groups are the TK (Tyrosine Kinases), TKL (Tyrosine Kinase-Like), STE (Ste- or MAP-kinases), CMGC (cyclin-dependent kinases, mitogen-activated kinases, glycogen synthase kinases and CDK-

like kinases), CK1 (casein kinase 1), AGC (protein kinase A, G and C) and CAMK (Calmodulin-dependent protein kinase). Overactivation of specific kinases can disturb cellular functions and indeed overactivation of various kinases is found in many tumours. Therefore, inhibition of specific kinases has gained much interest. Nowadays, 19 kinase inhibitors, either monoclonal antibodies or small organic molecules, are used therapeutically[2] against various malignancies and over 400 protein kinase inhibitors are currently under development[3].

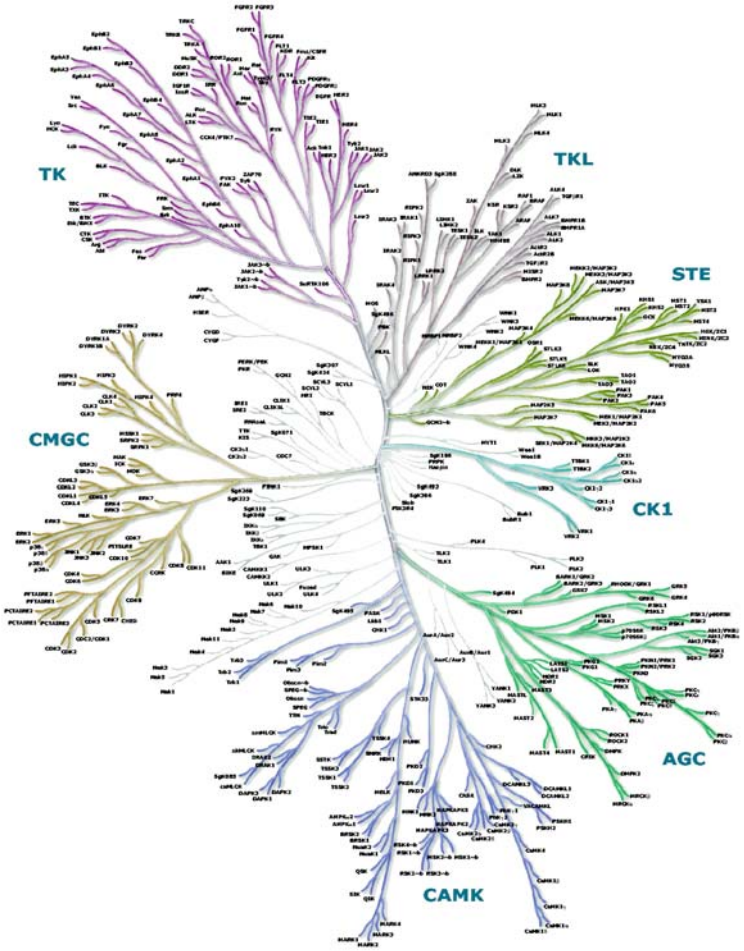


Figure 1: Dendrogram of human kinases classified in families

Sorafenib

Sorafenib (figure 2) is a small molecule multi-target kinase inhibitor that was discovered 1994 by high throughput screening technology. The screen was aiming at inhibition of the validated anti-cancer target Raf-1, a proto-oncogene serine and threonine kinase involved in the entry point of the MAPK and ERK signalling cascade (figure 3)[4].

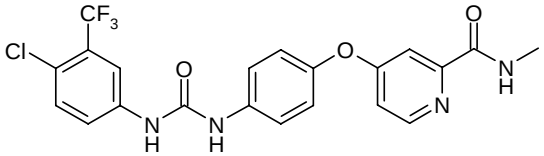


Figure 2: Chemical structure of sorafenib

Mitogen-activated protein kinase (MAPK) and extracellular-signal-regulated kinase (ERK) are part of a signalling system that connects membrane receptor binding to nuclear responses essential for cell proliferation. Hyperactivation of the MAPK/ERK pathway in tumour cells

can support immortalisation of the cell[5], growth-factor independent growth, insensitivity to growth-inhibitory signals, the ability to invade and metastasize, stimulate angiogenesis, avoid apoptosis and create resistance to therapeutic measures. The pathway is hyperactivated in 30% of all human solid tumours[6] . After its discovery it was found that sorafenib also inhibits *in vitro* at pharmacological concentrations, besides Raf-1, kinases involved in tumour angiogenesis. Figure 3 schematically represents the working mechanism of sorafenib in tumour and endothelial cells. In the tumour cell the overactivated kinase activity of Raf-1 is blocked, preventing further signal transduction *via* the MAPK/ERK pathway and blocking the avoidance of apoptosis. Due to a lack of oxygen and nutrition tumour cells excrete growth factors like hypoxia inducable growth factor cells which stimulate the kinases VEGFR (vascular endothelial growth-factor receptor) and PDGFR (plateled derived growth-factor receptor). In endothelial cells, the signal transduction of these membrane-anchored kinases is blocked by sorafenib, as well as Raf-1. In conclusion, sorafenib is able to block both tumour growth and angiogenesis.

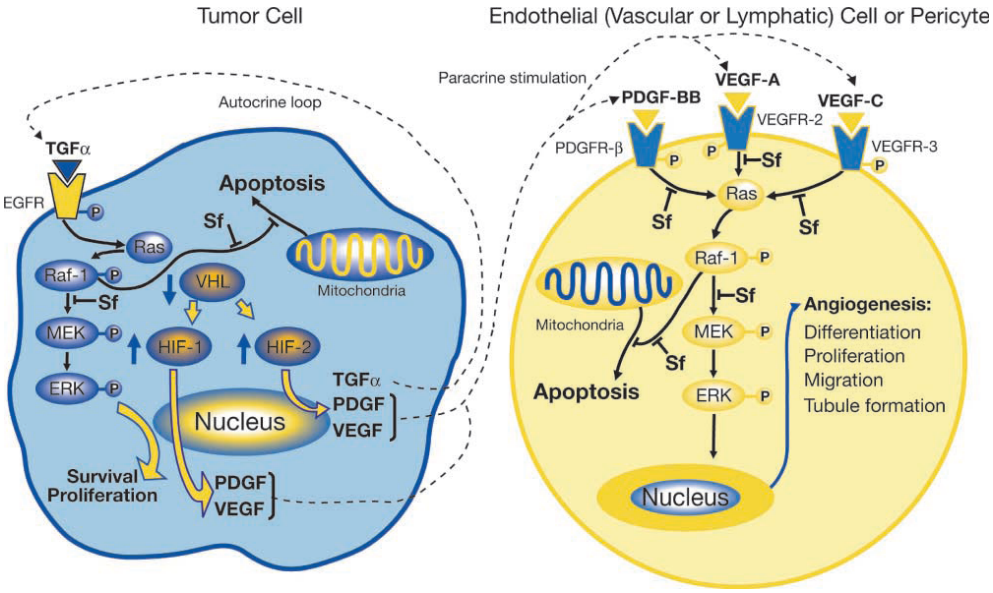


Figure 3: Sorafenib kinase inhibition mechanism in tumour and endothelial cell

Table 1 depicts the *in vitro* inhibition of sorafenib on a panel of kinases. Except for the above mentioned kinases, sorafenib also inhibits c-KIT and Flt-3 at pharmacological concentrations. C-Kit is a kinase involved in cell growth, which in healthy tissue is activated by autophosphorylation upon dimerization induced by stem cell factor. Mutations in the c-Kit gene can result in stem cell factor independent activation of this kinase, found in various cancers[7]. The kinase Flt-3 is involved in differentiation, growth and survival of hematopoietic stem cells and lymphoid and dendritic progenitor cells[8] and plays a key role in leukemogenesis.

Since its approval in 2004 by the FDA, sorafenib, product name Nexavar®, is clinically used for the treatment of hepatocellular carcinoma and advanced renal cell carcinoma. Furthermore numerous oncology related clinical trials concerning combination therapy including sorafenib are ongoing[9].

Kinase	Sorafenib, IC_{50} (µmol / L)
VEGFR-2	0.030-0.090
VEGFR-3	0.020-0.100
PDGFR-β	0.057-0.080
c-Kit	0.068
Flt-3	0.020-0.058
FGFR-1	0.58
EGFR	> 100
c-Met	> 100
IGFR-1R	> 100
Raf-1	0.006

Table 1: *In vitro* IC_{50}-concentrations of sorafenib on various kinases

Positron emission tomography

Positron emission tomography is a widely used non-invasive technique for the diagnosis of various diseases in the fields of cardiology, neurology and cancer. It depends on the emission of positrons by instable positron emitting radionuclides. These positrons will partially loose their kinetic energy by collisions while travelling a short distance (0.5 – 2 cm, depending on their energy) and finally annihilate with an electron of a surrounding atom to produce two gamma ray photons of 511 MeV in opposite direction. By positioning gamma cameras in a circle around the object of interest the approximate location of the positron emitting nuclides in this object can be determined because of the simultaneous detection of these photons. The location of the radionuclides then is the intersection point of the imaginary lines between the corresponding photons. Figure 4 depicts such an annihilation event of a positron, emitted from a carbon-11 atom, with an electron to produce two gamma photons that travel in opposite direction to form an imaginary line.

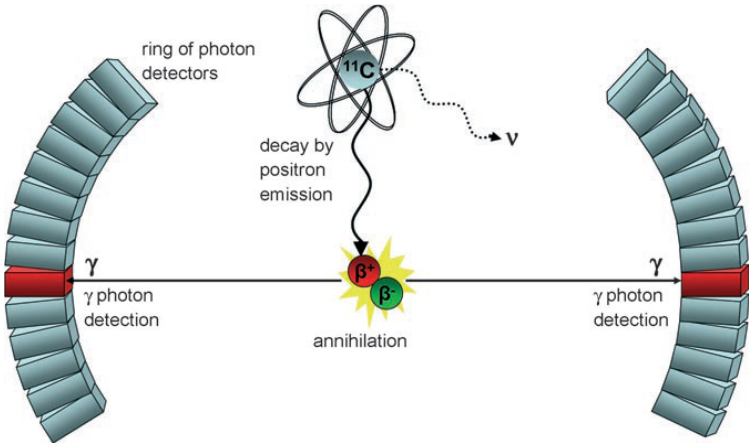


Figure 4: Schematic representation of the positron emission tomography principle

The large list of PET-isotopes includes small atoms (^{11}C, ^{13}N and ^{15}O) also found in biomolecules. This provides the possibility of labelling these biomolecules without interfering in their function. For labelling with a metal isotopes (e.g. ^{68}Ga, ^{89}Zr) the biomolecule must be equipped with a specific handle, a chelate, to form a 'stable' complex between this chelate and the metal atom on the biomolecule. ^{18}F can be introduced as a replacement of a ^{19}F atom in fluor containing compounds, given that this position in the molecule is chemically

accessible. For compounds lacking an accessible fluor atom, an alternative method for labelling with ^{18}F is to introduce a ^{18}F atom or a ^{18}F containing group to the molecule. The researcher must be alert that such modifications, especially in small organic compounds, might influence the pharmacodynamic properties of the analogue compared to the original compound. ^{124}I is used as a PET-isotope for the labelling of proteins as well as small molecules. Table 2 lists the above mentioned PET-isotopes and their half-lives.

Isotope	**$t_{1/2}$**
^{11}C	20.4 min
^{13}N	10 min
^{15}O	2 min
^{18}F	110 min
^{68}Ga	68 min
^{89}Zr	3.3 days
^{124}I	4.2 days

Table 2: Several PET-isotopes and their half-lives

By introduction of such positron emitting nuclides into biologically active compounds PET-imaging can be used to provide metabolic information that cannot be obtained by other imaging techniques such as MRI or CT.

The chemistry for the synthesis of labelled compounds is rather limited when compared to traditional organic chemistry. This is mostly caused by the small amount of readily available synthons and the relative short half-life the isotopes. After the end of bombardment of the target at a cyclotron (particle accelerator), the instable isotope is transferred to the synthesis cell in an inert carrier gas. Now it can be immediately used in the reaction or be converted to the desired (more reactive) synthon. Due to the short half-lives and the 'rule of thumb' that the total synthesis should take no longer than three isotope half-lives no complex synthons can be synthesized.

Positron emission tomography tracers

A powerful tool for diagnosis of tumours and their metastasis is positron emission tomography in combination with the glucose derivative ^{18}F-2-fluoro-2-deoxyglucose (^{18}F-FDG)[10]. FDG is taken up and phosphorylated by viable cells similar to glucose. However, since FDG lacks a hydroxyl group at the 2'-position, glycolysis cannot take place and therefore the phosphorylated sugar analogue accumulates in tissues with a high metabolic rate such as brain, heart and tumour tissue. This way viable tumours can be detected by positron emission tomography. As an example figure 5 shows the image of a patient with multiple lesions obtained by a [^{18}F]FDG-PET scan. Since high concentrations of [^{18}F]FDG result in the black dots in the image, it is clear that this patient has a large number of metastasis.

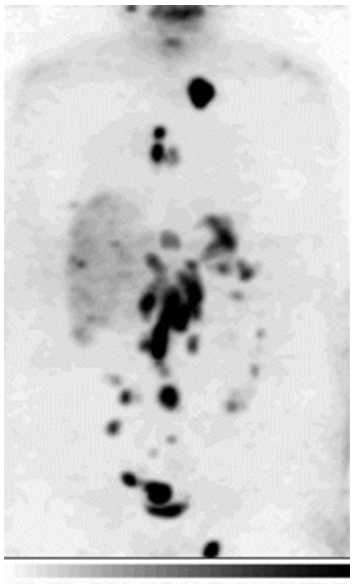


Figure 5: PET scan showing the

This same technique can also provide information on the response to therapy by performing a FDG-PET scan at the start of treatment (baseline) followed by a similar scan several weeks during therapy. Therapy effectiveness can be monitored and ineffective therapies can be adjusted in an early stage. Although this technique is widely used, it does not provide information on the chance of success of the therapy in advance. Predictive data can be obtained however, when before treatment the patient is treated with a radiolabelled PET-analogue of the pharmacon. By a positron emission tomography scan the biodistribution of the radiotracer is determined and co-localized tumour tissue and radioactivity indicates tumour sensitivity for this specific compound and is a positive signal for the start of treatment with the unlabelled pharmaceutical. This method is already clinically used for various pharmaceuticals, such as the EGFR inhibitors erlotinib[11] and gefitinib[12], used in the treatment of lung cancer. Since only 10-15% of the patients respond to therapy, patients are treated with the radiolabelled compound prior to treatment[13].

Sorafenib labelling strategy

Based on the successful use of several TKI-radiotracers for the prediction of therapy effectiveness, in this thesis sorafenib will be labelled with a PET-isotope. When considering the structure of sorafenib, there are multiple positions for PET isotope labelling. Since sorafenib contains a trifluoromethoxy-group, labelling with ^{18}F might be a possibility, however the introduction of such a ^{18}F labelled trifluoromethyl-group has yet to be studied intensively before its possible future use in the laboratory. Therefore, this study will focus on labelling with ^{11}C. This isotope has a half-life of 20.4 minutes, meaning that the total production (synthesis, purification and formulation) should take no longer then approximately one hour in order to end with enough activity for successfully performing a PET-scan. There are two positions in the molecule particularly suited for labelling with ^{11}C. The radioactive isotope can be introduced as methyl group on the terminal amide functionality (** in figure 6) or as urea carbon atom (* in figure 6). From a metabolic perspective, labelling at a central position in the molecule might be preferred since this prevents the radioactive isotope to be cleaved off the radiotracer. Introducing the label at the urea position would therefore expected to be more stable in comparison with labelling at the methylamide position, which can be susceptible to demethylases. On the other hand, the circulation of a labelled metabolite that still has a high affinity for its target may be problematic during imaging, since the measured signal then is derived from the parent tracer and its metabolite in an unknown ratio. In this perspective, labelling at the methylamide position might be preferred since demethylation would result in a large metabolite undetectable by PET and [^{11}C]methanol which will only result in a lower signal to noise ratio because of the homogeneous distribution throughout the body. The main known metabolites of sorafenib are the pyridine N-oxide and the glucuronidated metabolites and even 70-85 % is stable *in vivo* in the steady state[14]. Both labelling positions mentioned above will be evaluated in this thesis.

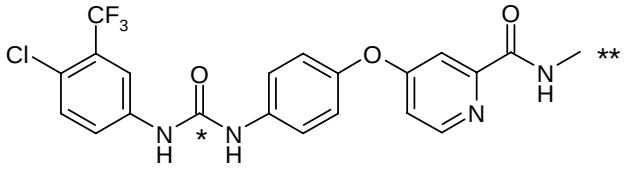
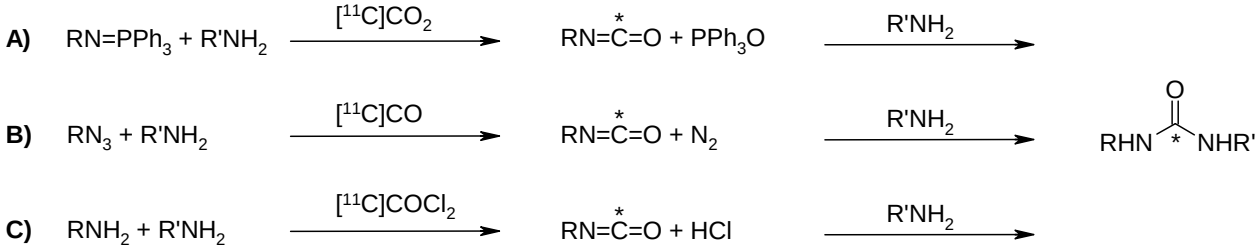


Figure 6: Structure of sorafenib with possible labelling positions marked with * and **

Due to the very short half-life of the positron emitting radionuclide C-11 of only 20.4 minutes, introduction of the label is preferably the last step in the synthesis of the tracer. Therefore, first precursor molecules will be synthesized which in a final reaction step will be labelled to form the biologically active compound sorafenib.

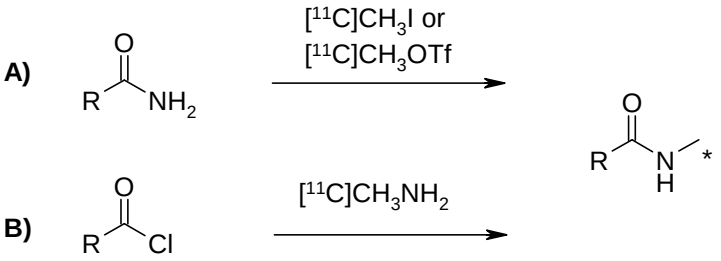
For carbon-11 labelling of an organic compound at a urea position multiple strategies are described in literature [15, 16, 17]. These reactions, depicted in scheme 1, require as radioactive synthons either $[^{11}C]CO_2$, $[^{11}C]CO$ or $[^{11}C]COCl_2$.



Scheme 1: Strategies for ^{11}C-labelling at a urea position

Reaction **A** in scheme 1 requires a phosphinimine (iminophospharane) to react with the synthon $[^{11}C]CO_2$, forming triphenylphosphine oxide and an intermediate isocyanate, which will react by nucleophilic addition of the free amine to form an unsymmetrical urea. This strategy has proven to be convenient for the synthesis of various unsymmetrical ureas. A big advantage is the relatively simple one-pot one-step-synthesis, since the radioactive precursor $[^{11}C]CO_2$ produced at the cyclotron is used without further conversion. A challenge is to overcome the low radiochemical yield when considering an aromatic (electron poor, less nucleophilic) amine[15].
Reaction **B** in scheme 1 is a metal (selenium, palladium or rhodium) mediated reaction between an azide, an amine and $[^{11}C]CO$. According to the proposed mechanism, the azide and carbon monoxide react while being complexed with the metal atom. Then, either by a nucleophilic attack of the amine the unsymmetrical urea is released from the complex or the intermediate isocyanate is released and is attacked by the amine to form the urea. This reaction will, like the reaction **A)** in scheme 1, also selectively result in the unsymmetrical urea product.
Finally, reaction **C** in scheme 1, the highly reactive $[^{11}C]COCl_2$ combined with two amines can be used to form an urea compound. Here, an isocyanate will be formed by a nucleophilic substitution by the first amine. This isocyanate will, by a nucleophilic addition of the second amine, form the desired urea. To minimize the amounts of symmetrical ureas, usually a two-step synthesis protocol is used. After the formation of the intermediate isocyanate in a relatively short reaction time, an excess of the second amine is added to form the unsymmetrical urea. Minimizing the amount of side-products this way requires much optimization of the reaction conditions. Another drawback are the technical difficulties for the reliable production of $[^{11}C]COCl_2$. Since our group has no production line for the synthesis of $[^{11}C]COCl_2$, this method will not be included in this research.

The other possibility for labelling is the introduction of the carbon-11 containing moiety at the methyl amide position. Two strategies can be followed, both depicted in scheme 2.

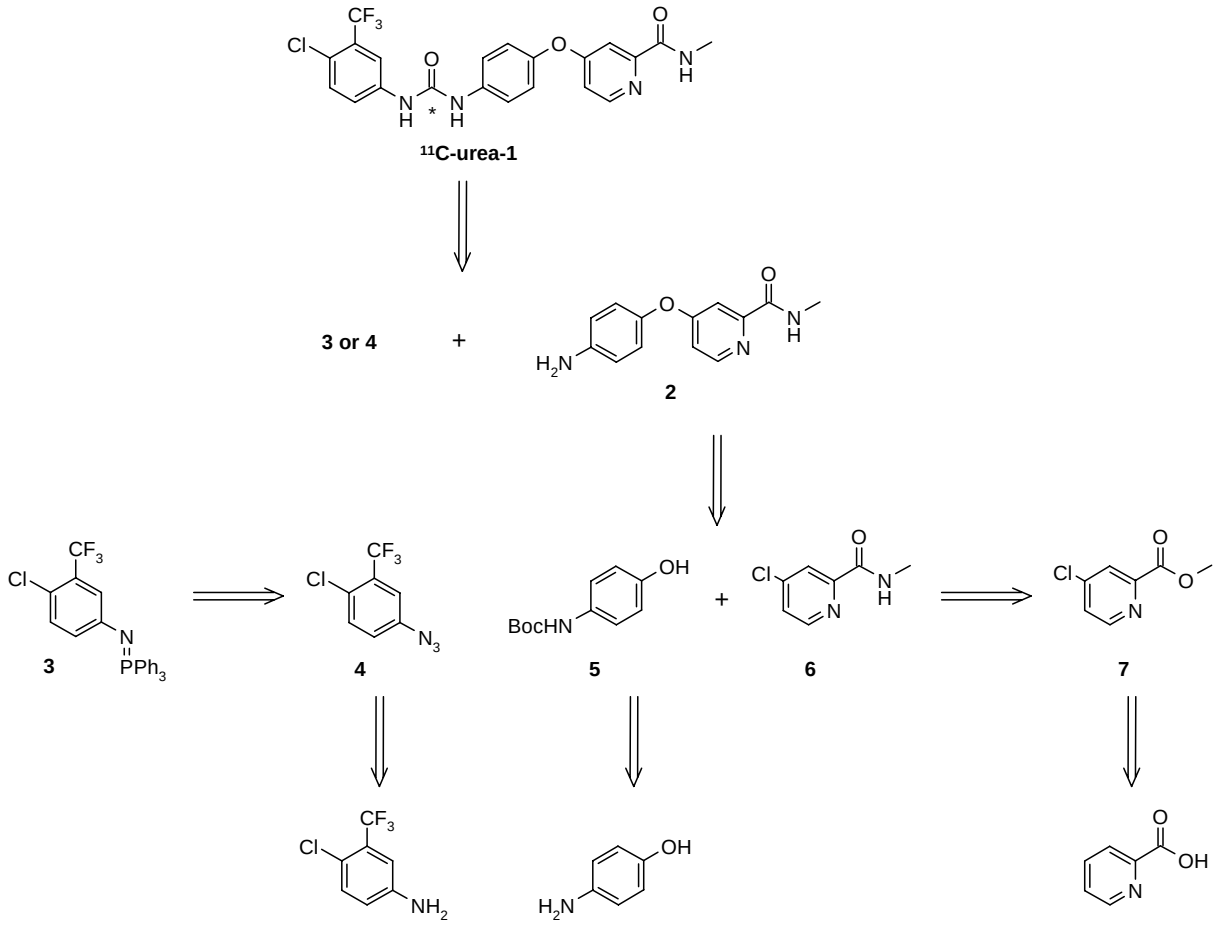


Scheme 2: Strategies for ^{11}C-labelling at a methylamide position

Reaction **A** in scheme 2 involves the synthon [^{11}C]CH_3I ([^{11}C]methyl iodide)[18] or [^{11}C]CH_3OTf ([^{11}C]methyl trifluoromethanesulfonate)[19]. Both synthons are used for the introduction of a [^{11}C]methyl group on e.g. a hydroxyl, amine or amide functionality. [^{11}C]methyl triflate is used as a highly reactive alternative for [^{11}C]methyl iodide, for methylation reactions that would otherwise require extreme temperatures or long reaction times. The reactivity of [^{11}C]methyl triflate is sufficient for labelling of primary amides at low temperature and has proven to be a successful strategy for various tracers[20, 21]. Drawbacks for the use of methyl triflate are its susceptibility for hydrolysis and the extra synthetic step for the conversion of methyl iodide to methyl triflate[22]. Therefore in this study first the methylation using methyl iodide will be explored. For reaction **B** in scheme 2 [^{11}C]methylamine[23] is used to react with the highly reactive acyl chloride. Difficulties using this procedure are the instability of acyl chlorides, which will therefore have to be prepared *in situ* prior to the labelling. More important, our group has no production line for [^{11}C]methylamine, which excludes this method on short term.

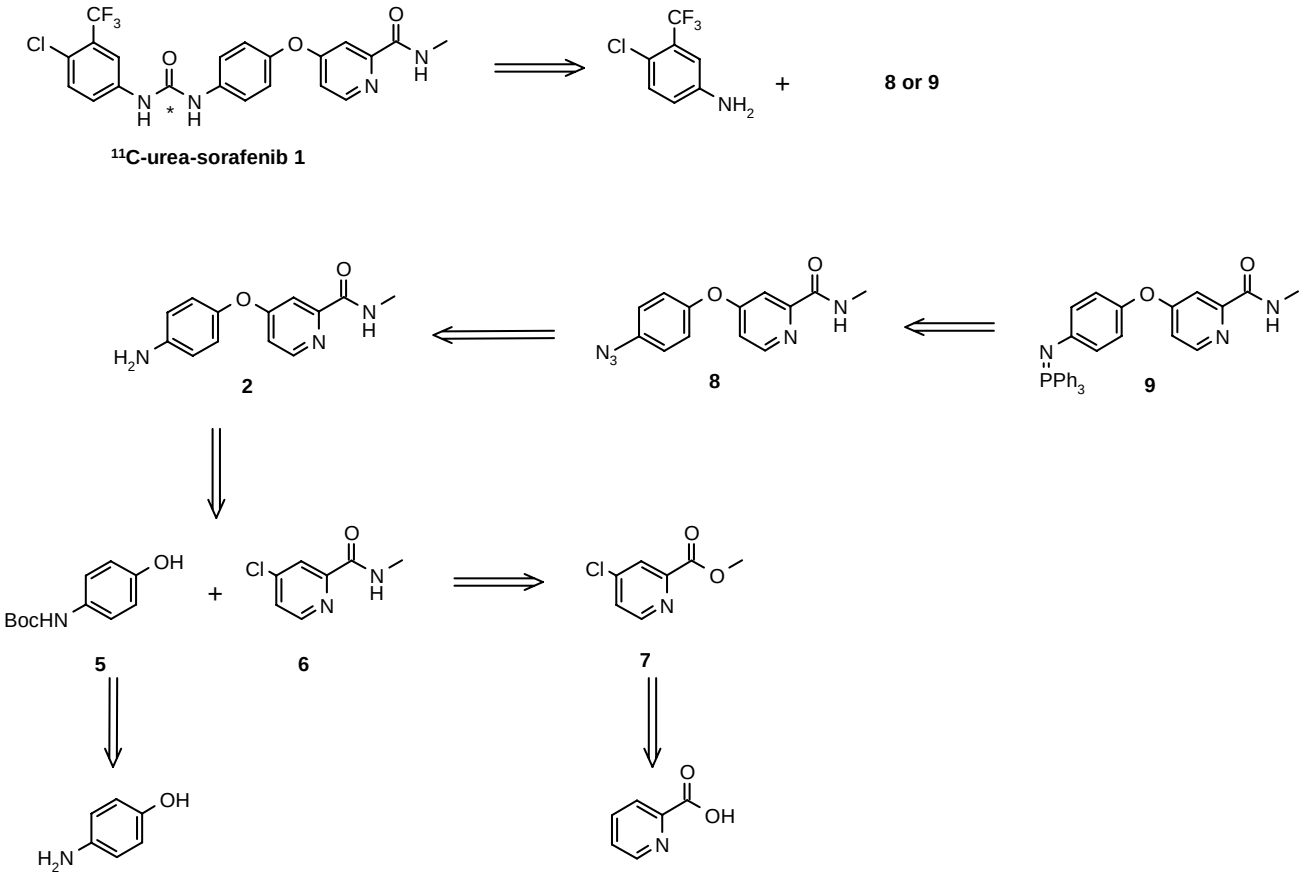
Synthetic approach

As mentioned above, labelling of sorafenib requires the synthesis of the right precursor molecules. Although sorafenib is an asymmetrical urea, the urea group by itself is symmetrical, so labelling at this position can be performed in two ways per pathway; R and R' in scheme 1 can be converted between the precursors. Therefore, the retrosynthesis of the precursors for labelling at the urea positions is depicted in two separate schemes, scheme 3 and 4.



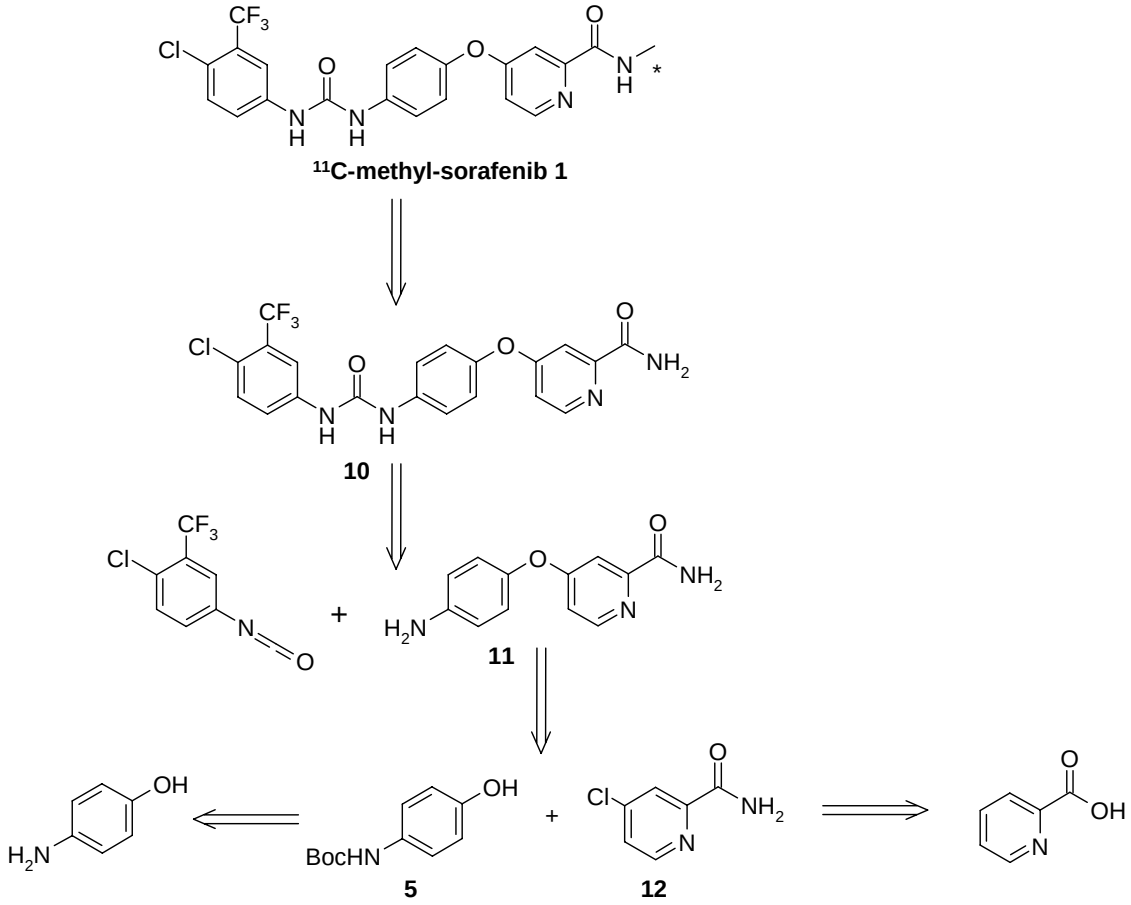
Scheme 3: Retrosynthetic pathway for the synthesis of [^{11}C]sorafenib with the radiolabel at the urea functionality

Scheme 3 and 4 show ^{11}C-urea-sorafenib **1** that will be synthesized from an amine with either a phosphinimine or an azide in two different pathways. Both these pathways will be explored in this study. The azide precursor molecules, **4** and **8**, are easily available since the phosphinimines precursor molecules **3** and **9** are synthesized from the corresponding azides by the Staudinger[24]. These azides can be directly synthesized from their corresponding amines[25]. 4-Chloro-trifuoromethoxy aniline is a commercially available compound whereas diarylether amine **2** has to be synthesized from Boc-aminophenol **5** and 4-chloro-N-methyl-pyridine carboxamide **6** by a Williamson ether synthesis. Compound **6** can be synthesized from the corresponding methyl ester **7** by a nucleophilic substitution of methylamine with methanol as leaving group. Methyl-4-chloro-pyridine carboxylate **7** can be prepared from the commercially available pyridine-2-carboxylic acid.



Scheme 4: Retrosynthetic pathway for the synthesis of [^{11}C]sorafenib with the radiolabel at the urea functionality

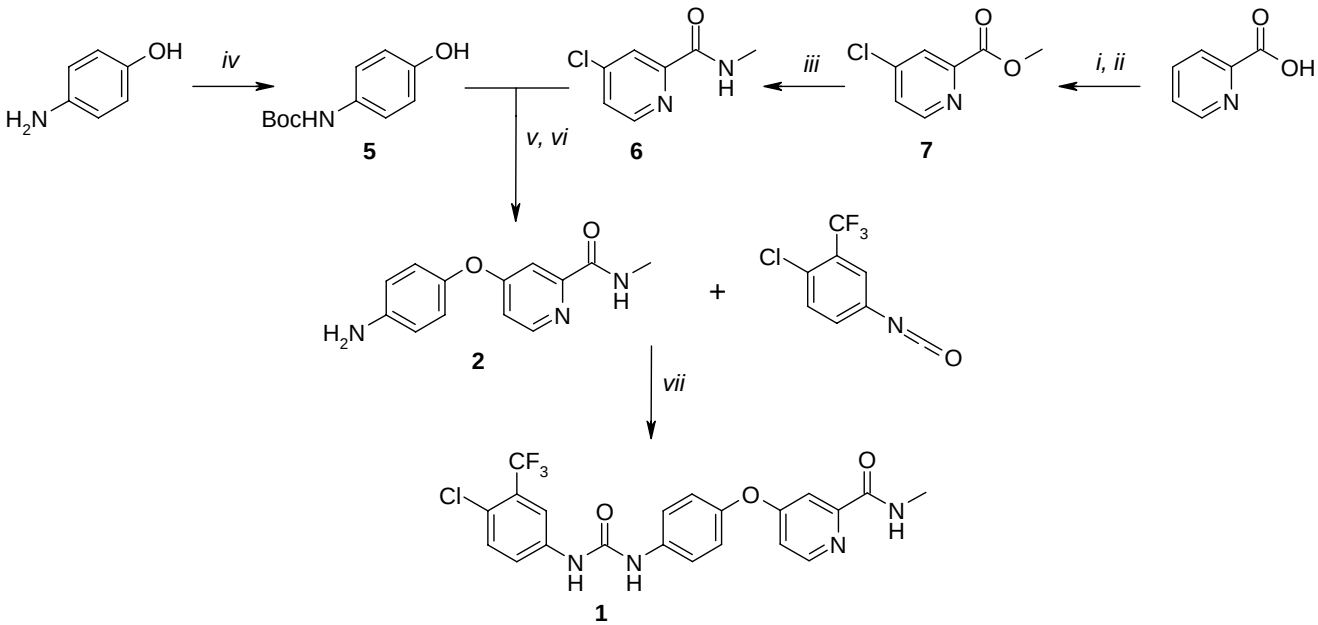
The retrosynthesis of ^{11}C-methyl-sorafenib **1** is depicted in scheme 5. For our chosen route to perform the labelling with either [^{11}C]methyl iodide or [^{11}C]methyl triflate, the precursor, compound **10**, only lacks the terminal methyl group in comparison with the desired product and is therefore called a desmethyl precursor. The urea functionality of this desmethyl precursor can be introduced by a reaction between the commercially available 4-chloro-3-trifluoromethyl phenyl isocyanate and diarylether amine **11**. Diarylether amine **11** will be synthesized by a Williamson ether synthesis between Boc-aminophenol **5** and 4-chloro-pyridine carboxamide **12**. Quenching of 4-chloro-pyridine-2-acyl chloride with gaseous ammonia will result in carboxamide **12.**



Scheme 5: Retrosynthesis pathway for the synthesis of [^{11}C]sorafenib with the label on the methylamide functionality

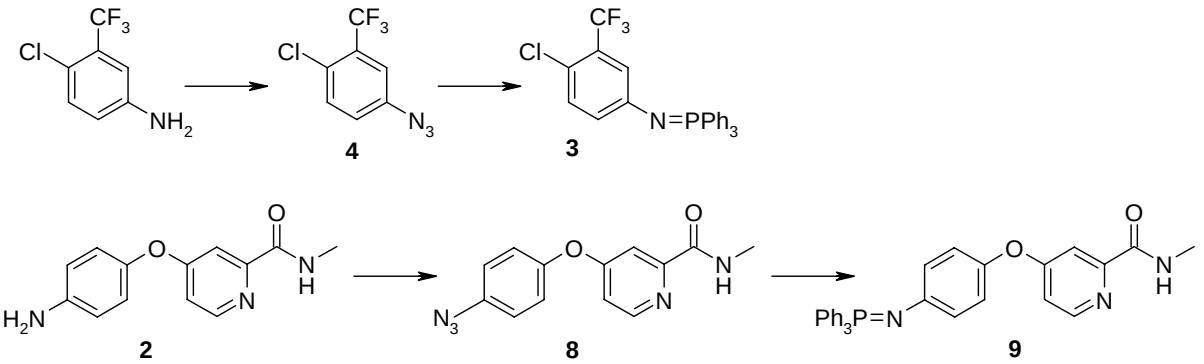
Results:

Prior to the synthesis of labelled ^{11}C-sorafenib, unlabelled sorafenib has to be synthesized to use as a reference in the analysis of the radiolabelling reactions. A full characterization of cold sorafenib can be performed (e.g. NMR, HPLC, MS), unlike with ^{11}C-sorafenib where, due to the nanomolar scale amounts, the analysis fully depends on HPLC with radiodetection. The synthesis of sorafenib (scheme 6) started with the conversion of pyridine-2-carboxylic acid to the intermediate acyl chloride in thionyl chloride and a catalytic amount of DMF. Quenching of this intermediate with methanol resulted in methyl ester **7**. The methyl ester was converted to methylamide **6** by a nucleophilic substitution with methylamine at room temperature in a THF/MeOH solution. Methylamide **7** reacts with Boc-protected aminophenol **5** in a Williamson reaction to afford diaryl ether amine **2** in 25 % yield. Besides the starting materials the reaction mixture contained dark insoluble particles that were no further analysed. The reaction conditions were varied to improve the yield but this, unfortunately, was not successful. A subsequent reaction of diarylether amine **2** with 4-chloro-3-trifluoromethoxyphenyl isocyanate in DCM resulted in cold sorafenib **1**, which precipitated out of DCM and was obtained in excellent purity after filtration. The overall yield of the synthesis of sorafenib was 8,4 % over 5 reaction steps.



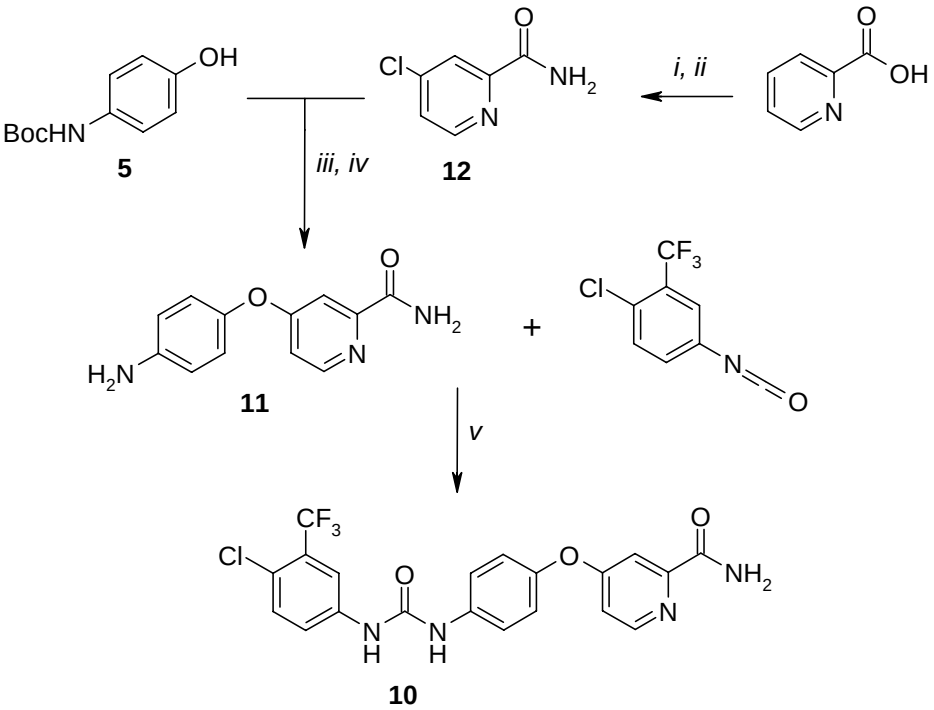
Scheme 6: Synthesis of cold sorafenib. Reagents and conditions: *(i)* $SOCl_2$, DMF, 16 h, 72 ˚C *(ii)* MeOH, 1 h, rt, 68 % *(iii)* CH_3NH_4Cl, D*i*PEA, MeOH/THF, overweekend, rt, 89 %. *(iv)* di-*tert*-butyl dicarbonate, H_2O / THF, $NaHCO_3$, 5 h, rt, quant. *(v)* NaOH, DMF, 16 h, 80 ˚C, 19 % *(vi)* TFA / DCM, 1h, rt, quant. *(vii)* DCM, 16 h, rt, 73 %

The synthesis of the phosphinimine and azido precursors (scheme 7) starts with the conversion of the corresponding amines, 4-chloro-3-trifluoromethyl amine and diarylether amine **2**, to azides, **4** and **8**, with tertiary butyl nitrite and trimethylsilyl azide. After purification by column chromatography azides **4** and **8** were obtained in excellent purity and good yields. These two azides are, besides being used as precursors in the rhodium-mediated labelling reactions, converted with triphenylphosphine to the corresponding phosphinimines **3** and **9**. The crude products were purified by crystallization.



Scheme 7: Synthesis of azido and phosphinimine precursors. Reagents and conditions: *(i)* *t*-$BuNO_2$, acetonitrile, 15 min., 0 ˚C *(ii)* $TMSN_3$, acetonitrile, 16 h, 0 ˚C → rt, 70 % *(iii)* PPh_3, diethylether, 16 h, rt, 87 % *(iv)* *t*-$BuNO_2$, acetonitrile, 15 min., 0 ˚C *(v)* $TMSN_3$, acetonitrile, 16 h, 0 ˚C → rt, 98 % *(vi)* PPh_3, diethylether, 16 h, rt, 64 %

The synthesis of the desmethyl precursor for the $[^{11}C]CH_3I$ or $[^{11}C]CH_3OTf$ methylation reaction (scheme 8) is comparable to the synthesis of cold sorafenib. The intermediate acyl chloride which was obtained by the conversion of pyridine-2-carboxylic acid with thionyl chloride and a catalytic amount of DMF was quenched with gaseous ammonia to form amide **12**. Williamson etherification of Boc-aminophenol **5** and pyridine amide **12** results in Boc-protected diarylether in the typical yield of 20 %. After deprotection of the Boc protecting group diaryl ether **11** was reacted with 4-chloro-3-trifluoromethoxyphenyl isocyanate to obtain the desmethyl precursor **10** in 45 % yield. This precursor was purified by silica column chromatography. The overall yield over three reaction steps was 4,5 %.

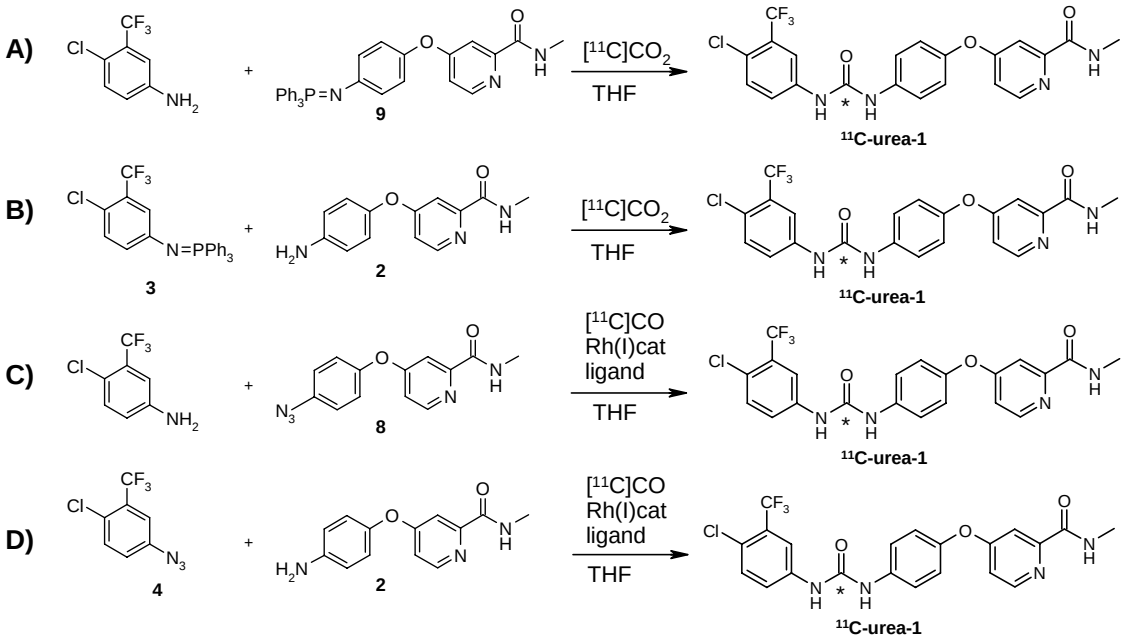


Scheme 8: Synthesis of desmethyl precursor for methylation. Reagents and conditions: *(i)* $SOCl_2$, DMF, 16 h, 72 ˚C *(ii)* NH_3, CH_2Cl_2, 2 h, rt, 53 % *(iii)* NaOH, DMF, 16 h, 80 ˚C, 19 % *(iv)* TFA / DCM, 1 h, rt, quant. *(v)* DCM / DMF, 16 h, rt, 45 %

Labelling results:

^{11}C-urea-sorafenib

In this study first the synthesis of ^{11}C-urea-sorafenib was explored by the reaction between ^{11}C-carbon dioxide and a phosphinimine[15]. The labelling protocol involves trapping of [^{11}C]CO_2 at -60 °C in a THF solution containing both precursors, a phosphinimine and the corresponding amine, and subsequent heating of the reaction solution to 60 °C for 6 minutes. A sample of this solution is then analysed by HPLC with UV and radio detection. A summary of these reactions is depicted in scheme 9.



Scheme 9: overview of synthesis routes possibly leading to ^{11}C-urea-sorafenib

The analytical HPLC chromatogram, figure 7, of a labelling with phosphinimine **9** and 4-chloro-3-trifluoromethyl aniline (reaction **A** in scheme 9) only shows two peaks from the precursor molecules in the UV chromatogram. The radioactivity chromatogram shows a peak of unreacted [^{11}C]CO_2 and another peak with a retention time of 7.1 minutes. Spiking the mixture with cold sorafenib, retention time 6.2 minutes, unfortunately revealed the desired product was not formed. Isolation of the formed compound by preparative HPLC showed that this unknown product was unstable, since [^{11}C]CO_2 was formed again. This indicates that the unstable intermediate isocyanate is possibly formed. Due to its electron withdrawing groups 4-chloro-3-trifluoromethyl aniline might be to little nucleophilic to react with this formed isocyanate to form ^{11}C-sorafenib.

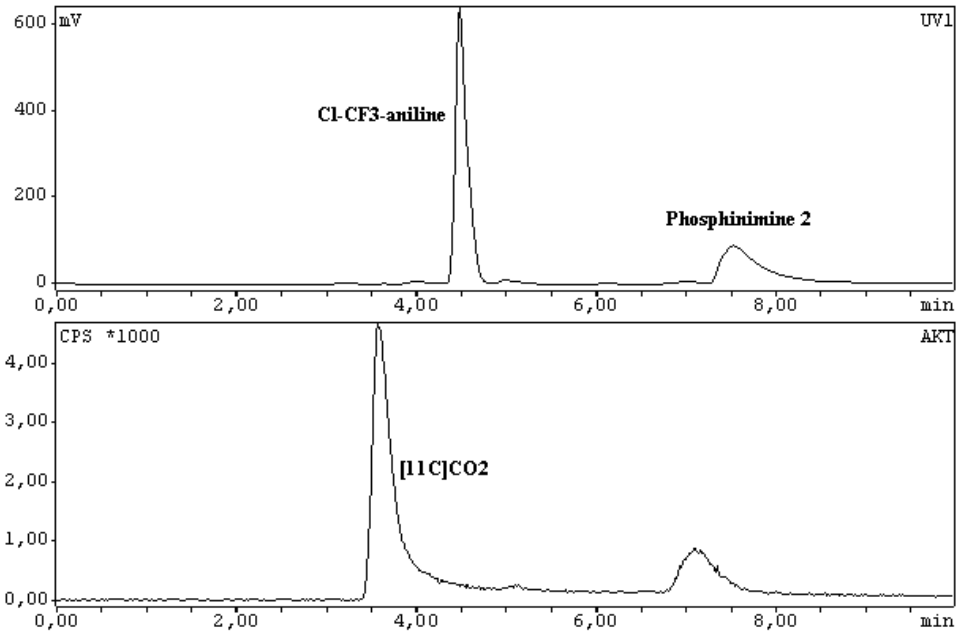


Figure 7: HPLC chromatogram of crude reaction mixture of reaction A in scheme 9

The low reactivity of 4-chloro-3-trifluoromethyl aniline might be circumvented by using 4-chloro-3-trifluorophenyl phosphinimine **3** (reaction **B** in scheme 9). Unfortunately the analytical HPLC chromatogram of this reaction only shows a radioactive peak belonging to $[^{11}C]CO_2$ and no other products were formed. The electron withdrawing groups of 4-chloro-3-trifluorophenyl phosphinimine **3** decrease its reactivity dramatically and thereby avoid the formation of an intermediate isocyanate required for formation of ^{11}C-urea-sorafenib.

The second approach to synthesize ^{11}C-urea-sorafenib involves the reaction between an azide and ^{11}C-carbon monoxide in the presence of rhodium(I) as a catalyst to form an intermediate isocyanate, followed by the reaction with an amine to form the desired product. The labelling starts with the production of $[^{11}C]CO$ by reduction of silica purified $[^{11}C]CO_2$ over a zinc column at 400 °C. In a micro-autoclave the formed $[^{11}C]CO$ is heated together with both precursors and catalyst in THF solution to 120 °C at 350 bar for 5 minutes. A sample of the reaction mixture is then analyzed by HPLC with UV and radio detection or ^{11}C-urea-sorafenib is isolated by semi-preparative HPLC. A summary of these reactions is depicted in scheme 9.

Initially this reaction was performed with azide **8** and 4-chloro-3-trifluoromethyl aniline (reaction **C** in scheme 9). Unfortunately, analysis of the reaction mixture by HPLC showed only trace amounts of product. This result confirms the low reactivity of 4-chloro-3-trifluoromethyl aniline due to the strong electron withdrawing groups.

Finally, ^{11}C-urea-sorafenib was synthesized with $[^{11}C]CO$ as radioactive synthon and 3-chloro-4-trifluoromethyl phenyl azide **4** and diarylether amine **2** as precursor molecules (reaction **D** in scheme 9). Analysis of the reaction mixture by analytical HPLC, figure 8, showed a major product peak at the activity channel with a retention time that corresponds with sorafenib. In contrast to the route using phosphinimines, here the intermediate 3-chloro-4-trifluoromethyl phenyl isocyanate is formed. Possible explanations for the successful synthesis are either a higher reactivity of carbon monoxide compared to carbon dioxide or a possible higher reactivity of an azide in comparison with a triphenyl phosphinimine. Also, the reaction conditions are more harsh. The peak area of ^{11}C-sorafenib on the activity channel

varies between 40 and 60 %. This radiochemical yield was determined by analytical HPLC. However, unreacted gaseous [^{11}C]CO can not be detected with this method, meaning that the radiochemical yield might be overestimated. This overestimation is corrected by measuring the activity in the product vial twice, immediately after the reaction and after removal of gaseous [^{11}C]CO by flushing 10 mL of air through the product solution. The difference in these two values implies the amount of gaseous [^{11}C]CO. After the successful synthesis of ^{11}C-urea-sorafenib the crude reaction mixture was purified by means of HPLC using an acetonitril / water mixture. The activity of the purified product was measured in a dose calibrator and from this a decay corrected yield was calculated starting from the end of synthesis. The isolated preparative yields in this reaction were much lower then was expected based on the analytical chromatograms combined with the amount of activity flushed away prior to the HPLC by the 10 mL air flush. This can be explained in several ways. At first, the HPLC-baseline on the activity channel never completely drops back to zero during the whole run. Probably the free [^{11}C]CO, [^{11}C]CO_2 or catalyst bound [^{11}C]CO gives a broad signal. Secondly, the 10 mL air flush might not remove all of the free volatile activity in the product vial. Upon loading the reaction mixture in the syringe for loading on the HPLC-loop with reduced pressure a significant amount of this activity might leave the solution into this gas phase in the syringe. Since only the solution is injected on HPLC actually less activity than expected might be injected. Table 3 depicts various reaction conditions explored in the synthesis of ^{11}C-urea-sorafenib.

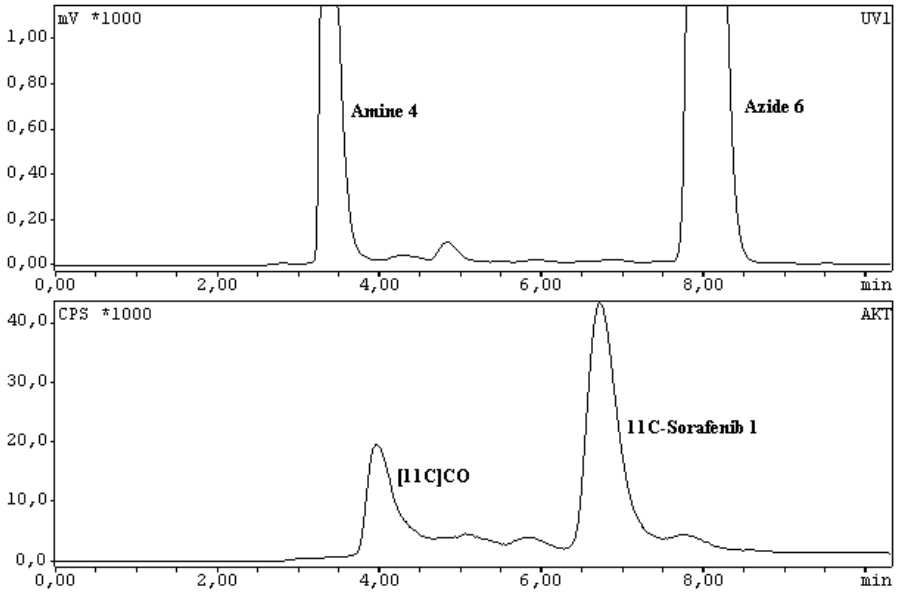


Figure 8: HPLC chromatogram of crude reaction mixture of reaction D in scheme 9

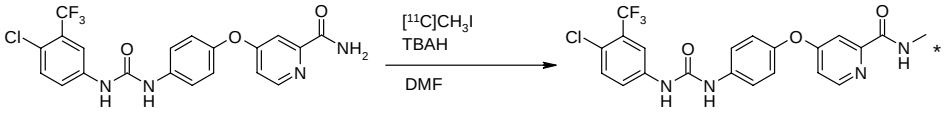
Entry	Azide **4** (mM)	Amine **2** (mM)	Rh-cat (mM)	Ligand (mM)	Flushed (%)	Isolated d.c. yield (%)
1	75	38	2.5	DPPE (5.5)	39.25 +/- 7.5	14.6 +/- 4.5
2[a]	75	38	2.5	PPh_3	51	8,2
3	75	38	0.6	DPPE (1.4)	46	10.5
4	75	100	2.5	DPPE (5.5)	47.1	20.9
5	75	154	2.5	DPPE (5.5)	40.3 +/- 5.1	27.1 +/- 9.3
6	75	38	2.5	PPh_3 (5.0)	18.3	28.9
7	75	154	2.5	PPh_3 (5.0)	15.25 +/- 3.4	53.2 +/- 2.4
8	75	76	2.5	PPh_3 (5.0)	25.5	31.9

Table 3: ^{11}C-urea sorafenib labelling results. Unless stated otherwise, Rh(cyclooctadiene chloride) dimer was used. [a]Wilkinson's catalyst ($RhCl(PPh_3)_3$ was used.

The most promising results were obtained using the rhodium cyclooctadiene chloride dimer catalyst, triphenylphosphine as a ligand, azide **4** and, in a high concentration of 154 mM, amine **2**. These conditions resulted in a high conversion of ^{11}[C]CO in non-volatile compounds, as seen by the low percentage of loss of activity by flushing, and only minimal amounts of side-products. Using these optimal conditions, entry 7 in table 3, the decay corrected radiochemical yield was 53.2 % (σ = 2.4, n = 4). Interestingly, using Wilkinson's catalyst [$RhCl(PPh_3)_3$] instead of [$Rh(cod)Cl]_2$ resulted in low isolated yields.

^{11}C-methyl-sorafenib

For the synthesis of ^{11}C-methyl-sorafenib a methylation of precursor **10** was done with methyl iodide, [^{11}C]CH_3I, under basic conditions. [^{11}C]CO_2 was trapped in a solution of $LiAlH_4$ in THF and the formed lithium methoxide salt is quenched with a 60 % HI solution in water at 130 °C. The formed methyliodide is distilled to the reaction vial containing a solution of desmethyl precursor **10** and tetrabutylammonium hydroxide in DMF. After heating the reaction vial to 80 °C for three minutes, a sample of this solution was analyzed by HPLC with UV and radio detection or the radiolabelled product was isolated by semi-preparative HPLC.



11 **10**

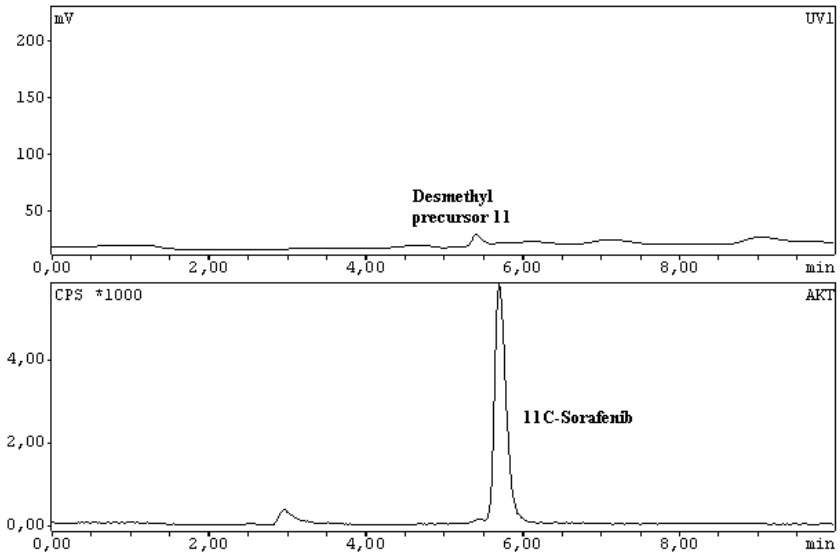


Figure 9: Chemical reaction and corresponding HPLC chromatogram of crude mixture after reaction

The analytical HPLC chromatogram of the crude reaction mixture (figure 9) clearly shows that ^{11}C-methyl-sorafenib, which has a retention time of 5.8 minutes, was the main radioactive product formed. The radiochemical yield of this reaction based on the activity channel of the analytical HPLC chromatograms varies between 60 and 90 %. After the successful synthesis of ^{11}C-methyl-sorafenib the crude reaction mixture was purified by means of HPLC using an acetonitril / water mixture. The activity of the purified product was measured in a dose calibrator and from this a decay corrected yield was calculated starting from the end of synthesis. The average decay-corrected radiochemical yield of this reaction was 58.8 % (n =3, σ = 12.2).

Formulation

Since both labelled ^{11}C-sorafenib products should finally be used *in vivo* both were formulated to make them suitable for intravenous injection. This means that the toxic organic solvents, acetonitril in our case, in the collected preparative HPLC fraction should be replaced by an non-toxic solvent. Herefore solid phase extraction chromatography was used; the purified product in acetonitrile / water solution was diluted with water and loaded on a Seppak-plus C18 column. The Seppak column was flushed with water to remove all traces of organic solvent and subsequently the trapped product was eluted from the Seppak column with ethanol. Since the product can not be administered in pure ethanol it was diluted with a 0.9 % NaCl and 0.4 % polysorbate solution in water till a final ethanol percentage of 9 %. Furthermore, to sterilize the product it was filtered over a sterile filter. For an *in vivo* experiment in mice, a maximum of 100 µL of the formulated product can be injected intravenously per mouse and 10-15 MBq should be injected for proper imaging. The 10 mL formulation must therefore have an activity between 1000 and 1500 MBq. For the ^{11}C-methylation as well as for the ^{11}C-urea labelling reaction this is achievable without using the highest amount of starting activity of 50 GBq (40 minutes, 30 µA). The methylation reaction takes about 40 minutes from end of bombardment at the cyclotron to the formulated product. Starting with an activity of 10 GBq (5 min, 30 µA) with an expected overall labelling yield of at least 50 %, 1.25 GBq would be formulated. The urea labelling reaction takes somewhat longer, approximately 50 minutes. Starting with 10 GBq would result, with an expected labelling yield of 50 %, in 900 MBq. These numbers show that the labelled product can be obtained in such a concentration using our formulation procedure which is suitable for the *in vivo* experiments. Alternative formulations can also be explored to obtain the labelled product

in less volume. For metabolite studies which will be performed in rats, a volume of 1 mL per rat can be injected. However, also more activity is required here for the metabolite analysis at different time-points.

Conclusions:

This report describes the successful synthesis of radiolabelled sorafenib at two different positions in the molecule by introduction of the PET-isotope ^{11}C.

At first, labelling of sorafenib by the method developed in our group that uses a phosphinimine to react with $[^{11}C]CO_2$ was explored. Unfortunately, despite various reaction conditions, this method was not successful. The electron withdrawing groups in 4-chloro-3-trifluoromethyl phenyl phosphinimine **3** lower the reactivity and prevent the formation of the essential intermediate isocyanate. An intermediate isocyanate is formed by the reaction between diaryl ether phosphinimine **9** and $[^{11}C]CO_2$. However, the amine functionality of 4-chloro-3-trifluoromethyl aniline is too electron poor to attack the isocyanate and form the desired urea product.

Secondly, labelling at the urea position was explored with success by a reaction with 3-chloro-4-trifluoromethyl phenyl azide **4**, diarylether amine **2**, $[^{11}C]CO$, rhodium(I)cyclooctadiene chloride dimer as catalyst and triphenylphosphine as a ligand in THF. The decay-corrected yield of this reaction based on preparative HPLC was 53.2 % (σ = 2.4, n = 4). Using 4-chloro-3-trifluoromethyl aniline and diaryl ether azide **8** as precursors and the same labelling protocol hardly any product was formed. An explanation for this is the low nucleophilicity of 4-chloro-3-trifluoromethyl aniline due to the electron withdrawing groups.

Finally, sorafenib labelling was established by methylation of desmethyl precursor **10** with $[^{11}C]CH_3I$ with tetrabutylammonium hydroxide in DMF. The product is obtained in good purity and high yield, 58.8 % (σ = 12.2, n = 3) after preparative HPLC.

Furthermore, both labelled products were successfully formulated by a solid phase extraction procedure using a Seppak plus C18 column. It should be noted that a 0.4 % polysorbate solution was essential to prevent the product from sticking to the Millipore filter. The final product was formulated in 11 mL 0.4 % polysorbate solution containing 9 % ethanol.

Future research:

The possibility of labelling sorafenib with carbon-11 at two distinct positions in the molecule is interesting in a metabolic perspective since different labelled metabolic products can be expected. Therefore, an *in vivo* metabolite study in rats will be performed. At various time points, blood and liver samples of rats injected with either one of the labelled compounds will be analyzed. Each peak on the radiogram other than the ^{11}C-sorafenib peak indicates a metabolic product. Such an experiment will provide valuable information to decide which labelled variant could be further developed.

In addition to a metabolite study an *in vivo* biodistribution study of both ^{11}C-labelled variants of sorafenib in nude tumour bearing mice will be performed. A suitable (overexpression of RAF-1, VEGFR, PDGFR, c-KIT or flt3) tumour cell line must be cultivated. The effect of cold sorafenib on the growth of this cell line can be explored *in vitro* to prove the sensitivity of these tumour cells towards sorafenib. When tumours in mice are grown and have reached the desired dimensions ^{11}C-sorafenib will be administered and distribution will be monitored in time by dynamic PET scanning. Tumour uptake will be then determined as well as possible differences between the two tracers.

Results of these two *in vivo* experiments provide valuable information supporting in the decision of which labelled ^{11}C-sorafenib variant should be further developed.

High tumour uptake of ^{11}C-sorafenib in the *in vivo* biodistribution study indicates that ^{11}C-sorafenib can indeed be used as a tracer for the prediction of therapy effectiveness. This should then be explored further in a patient study. Patients who might start with sorafenib treatment can volunteer to first undergo ^{11}C-sorafenib PET scan. The effect of the treatment combined with the information obtained by the PET-scan performed prior to treatment will finally show the true value of ^{11}C-sorafenib PET-scans in patients.

Finally, sorafenib, since it contains fluor atoms, might be labelled with the PET-isotope ^{18}F. Although the introduction of a [^{18}F]CF_3 is not yet described, this is explored in our group. Labelling of sorafenib with ^{18}F would, due to the longer half-life of ^{18}F in comparison with ^{11}C, make it possible to distribute this compound to other PET-centers.

Acknowledgements:

I could not have done this thesis on my own so therefore I would like to thank some people who supported me with my first exploration of the radiochemical field. First of all, Alex for giving me the opportunity to do my internship in the interesting field of kinase inhibitors and for his guidance and, together with Bert and Guus, for the supervision over this project. For introducing me to the field of carbon-11 radiochemistry I would like to thank Joost for giving an introduction and assisting me when using the CO-unit and Martien for his assistance with the use of the synthesis units in the GMP-lab. I would like to thank Bert for giving me the opportunity to work in his lab and finally everyone at the RadioNuclideCenter for the nice working environment.

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Experimental:

General:
All chemicals were used without further purification, unless otherwise stated. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 250 MHz NMR spectrometer; chemical shifts are expressed in parts per million (ppm) relative to the signal of the solvent (2.50 ppm for DMSO-d_6 and 7.27 ppm for $CDCl_3$ on the TMS scale). Thin-layer chromatography (TLC) was performed on Merck DC-alufolien, silica gel 60, F_{254}. Flash column chromatography was performed on silica gel 60 from J.T. Baker.
[^{11}C]CO_2 was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using an IBA Cyclone 18/9. Analytical HPLC was performed with a Jasco PU-1580 pump, an in-line Jasco UV-2075 UV detector (wavelength 254 nm), a homemade flow-through radioactivity detector and a C18 Grace Alltima column (5 µm, 4.6 x 250 mm). Semipreparative HPLC was performed with a Jasco PU-1587 preparative 50 mL/min pump, an in-line Jasco PU-1575 UV detector (wavelength 254 nm), a homemade flow-through radioactivity detector and a C18 Grace Alltima column (5 µm, 10 x 250 mm). Radioactivity was quantified with a VDC-405 dose calibrator (Veenstra Instruments, Joure, The Netherlands). All radiochemical reactions were carried out in homemade, remotely controlled devices.

4-chloro-3-trifuoromethyl phenyl azide (4):
To a solution of 4-chloro-3-trifluoromethyl aniline (400 mg, 2 mmol) in 5 mL acetonitrile *t*-butylnitrite (0.36 mL, 3 mmol) was added at 0 °C. Trimethylsilylazide (0.32 mL, 2.4 mmol) was added dropwise and the mixture was allowed to reach room temperature and stirred for 1h. After concentration *in vacuo*, purification was done by silica column chromatography (eluens: hexane). The product was obtained as yellow oil (312 mg, 70%). R_f (hexane): 0.80; IR: 2112.1 cm^{-1} (N_3); ^{1}H-NMR ($CDCl_3$): 7.53 (d, 1H, J = 8.6 Hz, Ar*H*), 7.37 (d, 1H, J_{meta} = 2.7 Hz, Ar*H*), 7.19 (dd, 1H, J_{meta} = 2.7 Hz, J = 8.6 Hz, Ar*H*) ppm; ^{13}C-NMR($CDCl_3$): 139.37, 132.82, 130.14, 129.63, 128.80, 128.04, 128.00, 124.45, 123.01, 120.10, 118.51, 118.43, 118.34, 118.26, 115.75; ^{19}F-NMR ($CDCl_3$): -63.16 (s, 3F, CF_3) ppm.

Methyl-4-chloro-pyridine carboxylate (7):
A solution of DMF (0.12 mL, 2.4 mmol) in 4 mL $SOCl_2$ was stirred for 10 minutes at 40 °C followed by the addition of picolinic acid (1.231 g, 10 mmol) in portions. The white picolinic acid slowly dissolved after turning green and the obtained purple solution was heated to 72 °C for 16h. The yellow mixture was concentrated and coevaporated with toluene (3x 10 mL). The orange residue was treated with 30 mL methanol and stirred at room temperature for 1h. The solution was concentrated *in vacuo*, dissolved in ethyl acetate and washed with subsequently saturated $NaHCO_3$ (2x 50 mL), H_2O (50 mL) and brine (2x 50 mL). After drying on Na_2SO_4 the filtered and concentrated residue was purified by silica column chromatography (eluens: hexane / ethyl acetate 1:1 *v/v*) to yield methyl ester **7** (1.172 mg, 68%) as off white crystals. R_f (hexane / ethyl acetate 1:1 *v/v*): 0.54; ^{1}H-NMR ($CDCl_3$): 8.65 (d, 1H, J = 5.2 Hz, Ar*H*), 8.15 (d, 1H, J = 2.0 Hz, Ar*H*), 7.51 (dd, 2H, J_{meta} = 1.9 Hz, J = 5.2 Hz, Ar*H*), 4.03 (s, 3H, CH_3) ppm; ^{13}C-NMR ($CDCl_3$): 164.6, 150.6, 149.2, 145.6, 127.2, 125.7, 53.23 ppm.

4-chloro-3-trifluoromethyl phenyl triphenylphosphinimine (3):
To a solution of 4-chloro-3-trifluoromethyl phenylazide (117 mg, 0.53 mmol) in ether (5 mL) was added triphenylphosphine (145 mg, 0.55 mmol). The green solution was stirred at room temperature for 16h. After concentration, the product was crystallized out of hot hexane / toluene to afford phosphinimine **3** as white crystals (180 mg, 87%). R_f (hexane / ethyl acetate

1:1 *v/v*): 0.79; ^{1}H-NMR ($CDCl_3$): 7.76-7.85 (m, 5H, Ar*H*), 7.49-7.64 (m, 10H, Ar*H*), 7.22 (d, 1H, *J* = 2.7 Hz, Ar*H*), 7.08 (d, 1H, *J* = 8.6 Hz, Ar*H*), 6.80 (dd, 1H, J_{meta} = 2.6 Hz, *J* = 8.6 Hz, Ar*H*); ^{13}C-NMR ($CDCl_3$): 150.55, 132.64, 132.48, 132.33, 132.28, 131.26, 130.77, 129.19, 129.01, 128.82, 128.47, 128.31, 128.19, 127.74, 126.43, 126.19, 125.48, 122.77, 122.69, 122.60, 122.52, 122.45, 122.36, 122.28, 122.20, 121.13, 118.74; ^{19}F-NMR ($CDCl_3$): -62.36 (s, 3F, C*F*$_3$) ppm.

4-chloro-N-methyl-pyridine carboxamide (6):
To a mixture of methylamine·HCl (2.7 g, 40 mmol) in THF (20 mL) was added methyl ester **9** BW011 (684 mg, 4 mmol) followed by D*i*PEA (5.2 g, 40 mmol) in THF (5 mL). The yellow mixture was stirred at room temperature for 72 h. The mixture was concentrated *in vacuo* and the residue was dissolved in ethyl acetate. After washing with brine (2x 50 mL), drying on Na_2SO_4, filtration and concentration compound **6** (606 mg, 89%) was obtained as a yellow oil. R_f (hexane / ethyl acetate 1:3 *v/v*): 0.55, ^{1}H-NMR ($CDCl_3$): 8.45 (d, 1H, *J* = 5.2 Hz, Ar*H*), 8.23 (d, 1H, J_{meta} = 2.0 Hz, Ar*H*), 8.00 (bs, 1H, N*H*), 7.45 (dd, 1H, J_{meta} = 2.1 Hz, *J* = 5.2, ArH), 3.0 (d, 3H, *J* = 5.1 Hz, C*H*$_3$); ^{1}H-NMR ($CDCl_3$): 163.73, 151.41, 148.98, 145.96, 126.25, 122.82, 26.21 ppm.

Boc-aminophenol (5):
To a solution of 4-aminophenol (4.0 g, 37 mmol) in H_2O / THF (100 mL, 1:1 *v/v*) Boc_2O (8.8 g, 40.4 mmol) and $NaHCO_3$ (3.4 g, 40.4 mmol) were added at 0 °C. The purple bubbling solution was allowed to warm to room temperature and stirred for 5h. The solution was concentrated *in vacuo* and the residue was dissolved in ethyl acetate. The organic solution was washed with subsequently water (50 mL), 1 M $KHSO_4$ (2x 50 mL), and brine (50 mL). After drying on Na_2SO_4, filtration and concentration, compound **7** (10.7 g, quant.) was obtained as a white solid. R_f (hexane / ethyl acetate 2:1 *v/v*): 0.57; ^{1}H NMR (DMSO-D_6): 9.01 (s, 1H, O*H*), 8.93 (bs, 1H, N*H*), 7.24 (d, 2H, *J* = 8.8 Hz, Ar*H*), 6.66 (d, 2H, *J* = 8.8 Hz, Ar*H*), 1.46 (s, 9H, C*H*$_3$); ^{13}C-NMR (DMSO-D_6): 152.98, 152.48, 130.98, 120.03, 114.97, 78.37, 28.17 ppm.

Boc-protected diaryl ether methylamide:
To a solution of boc-protected aminophenol **5** (114 mg, 0.5 mmol) and 4-chloro-N-methyl pyridine carboxamide (85 mg, 0.5 mmol) in DMF (5 mL) was added NaOH (21 mg, 0.53 mmol). The mixture was heated at 80 °C for 16h until TLC TLC showed no further conversion of the starting materials. The cooled solution was concentrated *in vacuo* and purification was done by silica column chromatography (eluens: hexane / ethyl acetate 3:2 *v/v*). The product was obtained as a colorless oil (32 mg, 19 %). R_f (hexane / ethyl acetate 2:1 *v/v*): 0.12; ^{1}H-NMR ($CDCl_3$): 8.41 (d, 1H, *J* = 5.7 Hz, Ar*H*), 8.15 (bs, 1H, N*H*), 7.76 (d, 1H, J_{meta} = 2.5 Hz, Ar*H*), 7.48 (dd, 2H, J_{meta} = 2.1 Hz, *J* = 6.8 Hz, Ar*H*), 7.09 (d, 2H, *J* = 2.2 Hz, Ar*H*), 6.99 (dd, 1H, J_{meta} = 2.6 Hz, *J* = 5.7 Hz, Ar*H*), 6.62 (bs, 1H, N*H*), 3.06 (d, 3H, *J* = 5.1 Hz, C*H*$_3$), 1.58 (s, 9H, C*H*$_3$); ^{13}C-NMR ($CDCl_3$): 166.89, 164.29, 152.82, 151.98, 149.27, 148.75, 136.36, 121.43, 120.35, 113.94, 110.39, 80.73, 28.34, 26.21 ppm.

Diaryl ether amine (2)
A solution of boc protected diaryl ether methylamide (250 mg, 0.73 mmol) was dissolved in DCM / TFA (6 mL 1:1 *v/v*). After stirring for 1 h, the red solution was concentrated *in vacuo* and dissolved in ethyl acetate (50 mL). The organic solution was washed with aq. sat. $NaHCO_3$ (50 mL), dried on Na_2SO_4 and concentrated *in vacuo*. Purification was done by silica column chromatography (eluens: ethyl acetate / hexane 2:1 *v/v*) and compound **2** was obtained as a colorless oil (160 mg, 90%). ^{1}H-NMR ($CDCl_3$): 8.37 (d, 1H, *J* = 5.7 Hz, Ar*H*), 8.05 (bs, 1H, N*H*), 7.71 (d, 1H, J_{meta} = 2.5 Hz, Ar*H*), 6.94 (m, 3H, Ar*H*), 6.77 (m, 2H, Ar*H*),

3.83 (bs, 2H, N*H*$_2$), 3.04 (d, 3H, *J* = 5.1 Hz, C*H*$_3$), ^{13}C-NMR (DMSO-D$_6$): 165.71, 163.66, 152.40, 150.32, 149.48, ?136?, 121.94, 119.86, 114.21, 108.83, 25.96 ppm. ESI-MS: calculated for $C_{13}H_{13}N_3O_2$: 243.27, found: *m/z* 244.11 $[(M+H)]^+$, 266.09 $[(M+Na)]^+$.

Diaryl ether azide (8):
A solution of boc-protected diaryl ether (160 mg, 0.47 mmol) in DCM / TFA (6 mL, 1:1 *v/v*) was stirred for 1 h at room temperature. After concentrating *in vacuo* the yellow oil was dissolved in acetonitrile (2 mL) and cooled to 0 °C. *t*-Butyl nitrite was added dropwise (0.09 mL, 0.7 mmol) and the yellow solution was stirred for 15 minutes. Upon addition of trimethylsilyl azide (0.08 mL, 0.6 mmol) the mixture was allowed to reach room temperature and stirred for 16 h. The solution was concentrated *in vacuo* and purified by silica column chromatography (eluens: hexane / ethyl acetate 1:1 *v/v*). Azide **8** (124 mg, 98%) was obtained as an off-white solid. IR: 2102.5 cm^{-1} (N_3); ^{1}H-NMR ($CDCl_3$): 8.2 (d, 1H, *J* = Hz, Ar*H*), 8.8 (bs, 1H, N*H*), 7.5 (s, 1H, Ar*H*), 6.9 (s, 4H, Ar*H*), 6.8 (d, 1H, *J* = Hz, Ar*H*), 2.8 (d, 3H, J = Hz, CH_3) ppm; ^{13}C-NMR ($CDCl_3$): 166.15, 164.39, 152.41, 150.67, 149.75, 137.54, 122.30, 120.77, 114.10, 110.05, 77.66, 77.15, 76.64, 26.12 ppm.

Diarylether phosphinimine (9):
To a yellow solution of diarylether azide **8** (100 mg, 0.56 mmol) in dichloromethane (10 mL) triphenyl phosphine (154 mg, 0.59 mmol) was added. After stirring at room temperature overnight the solution was concentrated *in vacuo* to yield a yellow solid. Purification was done by silica column chromatography (eluens: ethyl acetate) to yield phosphinimine **9** (171 mg, 91%) as an off-white solid. R_f (ethyl acetate): 0.53; ^{1}H-NMR ($CDCl_3$): 8.33 (d, 1H, *J* = 5.6 Hz, Ar*H*), 8.03 (d, 1H, *J* = 4.7 Hz, N*H*), 7.48-7.85 (m, 16H, Ar*H*), 6.75-6.93 (m, 5H, Ar*H*), 3.04 (d, 3H, *J* = 5.1 Hz, C*H*$_3$) ppm. ^{13}C-NMR ($CDCl_3$): 167.31, 164.83, 152.06, 149.42, 145.04, 133.60, 132.73, 131.53, 129.93, 128.75, 124.53, 121.08, 113.63, 110.16, 26.10 ppm.

4-chloro-pyridine carboxylamide (12):
After a solution of DMF (0.13 mL, 2.5 mmol) in $SOCl_2$ (5.95 g, 50 mmol) was stirred for 15 min at 45 °C, picolinic acid (1.231 mg, 10 mmol) was added in portions. The white picolinic acid particles slowly dissolved after turning from green to purple. The mixture was heated to 72 °C and stirred overnight. The orange slurry was allowed to reach room temperature, concentrated *in vacuo* and coevaporated with dichloromethane (2x 10 mL). Through the suspension in dichloromethane (25 mL) gaseous NH_3 was bubbled for 1 h. After concentrating the mixture *in vacuo* the residue was dissolved in dichloromethane (150mL) and washed with saturated $NaHCO_3$ (50 mL). The aqueous phase was extracted twice with dichloromethane (50 mL) and the collected fractions were dried on Na_2SO_4 and filtered. After concentrating the solution, purification was done by silica column chromatography (hexane / ethyl acetate 1:2 *v/v*) to afford compound **12** as an off-white solid (832 mg, 53 %). R_f (ethyl acetate / hexane 3:1): 0.62; ^{1}H-NMR ($CDCl_3$): 8.53 (d, 1H, *J* = 5.2 Hz, Ar*H*), 8.27 (d, 1H, J_{meta} = 1.9 Hz, Ar*H*), 7.86 (bs, 1H, N*H*), 7.51 (dd, 1H, J_{meta} = 2.1, *J* = 5.3 Hz, Ar*H*), 5.91 (bs, 1H, N*H*); ^{13}C-NMR ($CDCl_3$): 165.56, 150.99, 149.18, 145.99, 126.67, 123.21 ppm.

Boc-protected diaryl ether amide:
To a yellow solution of 4-chloro-pyridine carboxamide **12** (250 mg, 1.6 mmol) and boc-aminophenol **7** (480 mg, 2.3 mmol) in 10 mL DMF were added NaOH pallets (192 mg, 4.8 mmol). The purple mixture was heated to 80 °C and stirred overnight. After concentrating the mixture *in vacuo* the residue was dissolved in ethyl acetate and washed with brine (1x 50 mL). The organic solution was dried on Na_2SO_4, concentrated *in vacuo* and purified by silica column chromatography (hexane / ethyl acetate 1:1 *v/v*) to afford the product as a white solid

(100 mg, 19 %). R_f (hexane / ethyl acetate 1:1 *v/v*): 0.24; ^{1}H-NMR ($CDCl_3$): 8.45 (d, 1H, *J* = 5.6 Hz, Ar*H*), 7.97 (bs, 1H, N*H*), 7.75 (d, 1H, J_{meta} = 2.5 Hz, Ar*H*), 7.48 (d, 2H, *J* = 8.9 Hz, Ar*H*), 7.05 (m, 3H, Ar*H*), 6.64 (bs, 1H, N*H*), 1.58 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$): 167.37, 165.25, 152.80, 151.00, 149.25, 148.51, 136.59, 121.41, 120.42, 114.45, 111.06, 80.84, 28.35 ppm.

Sorafenib (1):
To a solution of diaryl ether amine **2** (49 mg, 0.21 mmol), in DCM (3 mL) a solution of 4-chloro-3-trifluoromethyl phenyl isocyanate (47 mg, 0.21 mmol) in DCM (3 mL) was added. After stirring at room temperature for 16 h the white precipitate was separated from the DCM solution by filtration and the residue was washed with DCM. After drying *in vacuo* 67 mg sorafenib was yielded as a white solid (73%). R_f (ethyl acetate / methanol 95:5): 0.56; ^{1}H-NMR (DMSO-D6): 9.23 (bs, 1H, NH), 9.01 (bs, 1H, N*H*), 8.76 (d, 1H, *J* = 4.9 Hz, N*H*), 8.51 (d, 1H, *J* = 5.6 Hz, Ar*H*), 8.13 (d, 1H, J_{meta} = 2.2 Hz, Ar*H*), 7.62 (m, 4H, Ar*H*), 7.39 (d, 1H, J_{meta} = 2.5 Hz, Ar*H*), 7.18 (m, 3H, Ar*H*), 2.79 (d, 3H, *J* = 4.8 Hz, CH_3) ppm. ^{13}C-NMR (DMSO-D6): 165.93, 163.77, 152.43, 150.35, 147.83, 139.31, 137.02, 131.98, 126.84, 126.45, 124.97, 123.11, 122.31, 121.41, 120.62, 116.86, 113.99, 108.66, 25.96 ppm; ^{19}F-NMR (DMSO-D6): 61.44 (s, 3F, CF_3) ppm. ESI-MS: calculated for $C_{21}H_{16}F_3ClN_4O_3$: 464.83, found: *m/z* 465.09 $[(M+H)]^+$.

Sorafenib desmethyl precursor (10)
A solution of Boc-protected diaryl ether amide (220 mg, 0.67 mmol) in DCM / TFA (6 mL 1:1 *v/v*) was stirred for 1h at room temperature. The orange solution was concentrated and dissolved in ethyl acetate. The organic solution was washed with aq. sat. $NaHCO_3$ () and the aqueous phase was extracted once with ethyl acetate. The collected ethyl acetate fractions were pooled and dried on Na_2SO_4. The Boc-deprotected compound, 69 mg (45%), was obtained in good purity after silica column chromatography (hexane / ethyl acetate 1:3). This product (69 mg, 0.3 mmol) was dissolved in DCM and 4-chloro-3-trifluoromethyl phenyl isocyanate (73 mg, 3.3 mmol) was added. After stirring overweekend at room temperature the concentrated residue was taken up in silica and purified by silica column chromatography (toluene / ethyl acetate 1:1) to yield sorafenib desmethyl precursor **10** as a white solid (120 mg, 40% over these two steps). R_f (ethyl acetate / MeOH 98:2): 0.54; ^{1}H-NMR (DMSO-D6): 9.23 (s, 1H, N*H*), 9.00 (s, 1H, N*H*), 8.52 (d, 1H, *J* = 5.6 Hz, ArH), 8.13 (m, 2H, N*H,* Ar*H*), 7.64 (m, 5H, N*H*, 4x Ar*H*), 7.40 (d, 1H, J_{meta} = 2.5 Hz, Ar*H*), 7.17 (m, 3H, 3x Ar*H*) ppm. ^{13}C-NMR (DMSO-D6, 125 MHz): 166.4 (*C*=O), 165.9 (*C*=O), 153.1, 152.9 (Ar*C*), 150.9 (Ar*C*H), 148.3, 139.7, 137.5 (Ar*C*), 132.4 (Ar*C*H), 127.1 (q, Ar*C*CF_3), 123.6 (Ar*C*H), 123.3 (q, *C*F_3), 122.8 (Ar*C*), 121.9, 120.9 (Ar*C*H), 117.3, 114.6, 109.2 (Ar*C*H) ppm. ESI-MS: calculated for $C_{20}H_{14}N_4O_3$: 450.81, found: *m/z* 451.08 $[(M+H)]^+$.

Radiosynthesis:
^{11}C-urea-sorafenib 1
In a helium stream (20 mL / min), [^{11}C]CO_2 was passed through a silica column. The first 50 MBq eluting from this column were discarded and subsequently [^{11}C]CO_2 was collected for three minutes. This [^{11}C]CO_2 was passed through a Zink-column at 400 °C. After passing through a ascarite column, the [^{11}C]CO was collected in a precooled small silica containing loop in liquid nitrogen. The concentrated [^{11}C]CO was then released by heating, collected in a micro-autoclave and a solution of 4-chloro3-trifluoromethyl phenyl azide **4** (6.7 mg, 30 µmol), diaryl ether amine **2 (**15 mg, 61 µmol , Rh(I)chloro(1,5-cyclooctadiene) (0.5 mg, 1 µmol) and PPh_3 (0.5 mg, 2 µmol) in 400 µL distilled THF was added. The reaction mixture

was pressurized to 350 bar and heated at 120 °C for 5 minutes. The reaction mixture was collected, purified by preparative HPLC and analyzed by analytical HPLC. R_t (C18 alltima, 60:40 acetonitril / water + 0.1 % TFA, 1 mL/min, 254 nm): 8.5 minutes, R_t (C18 platinum, 60:40 acetonitril / water + 0.1 % TFA, 1 mL/min, 254 nm): 6.2 minutes. Co-injection of cold sorafenib confirmed the identity of the labelled compound.

^{11}C-methyl-sorafenib

In a helium stream (10 mL / minute), [^{11}C]CO_2 was bubbled through 100 µL 0.1 M $LiAlH_4$ solution in THF. The solution was dried by heating the reaction vial to 130 °C. To the residue was added 2.2 mL 60 % hydrogen iodide solution in water and the formed [^{11}C]CH_3I was distilled through a sicapent column to a second reaction vial containing a solution of sorafenib desmethyl precursor **11** (1 mg, 2.2 µmol) and TBAH (1 µL, 2 µmol, 60 % solution in water) in 100 µL DMF. This solution was heated at 80 °C for 3 minutes. The reaction mixture was purified by preparative HPLC and analyzed by analytical HPLC. R_t (C18 alltima, 60:40 acetonitril / water + 0.1 % TFA, 1 mL/min, 254 nm): 8.5 minutes, R_t (C18 platinum, 60:40 acetonitril / water + 0.1 % TFA, 1 mL/min, 254 nm): 6.2 minutes. Co-injection of cold sorafenib confirmed the identity of the labelled compound.

Supporting information

Urea labelling protocol:

Date: Hot Cell: Act:

Preparations:
Hot Cell 9:
Fill two beakers with hot water
Fill small nitrogen dewar
Zn-column 400 °C, micro-autoclave 120 °C
Dissolve precursors, Rh-cat and PPh_3 in distilled THF, 400 µL
CO-trap in liquid helium Dewar
Hot Cell 8:
Connect alltima C18 prep column, eluens 60:40 AcN / H2O + 0.1 % TFA
Flush tubing and column with eluens
Prepare 1 mL syringe with AcN / H2O
Product flacon with exhaust needle
Hot cell 8 buttons: HPLC, compressed air

Reaction:
Connect closed MOBI to the system in hot water
Switch valve 1 from B to A and back after 5 sec
Open MOBI (T =) and wait till 50 MBq is trapped in ascarite (T =)
Switch valve 1 to A
Collect activity for three minutes, then valve 1 to B, valve 2 to stop, valve 3 stop
Move CO from CO trap to hot water beaker
Open valve 2 for 20 seconds and close (V2 stop → silica → stop)
Now that the activity is in autoclave inject THF solution on the loop (valve 4 position B)
Valve 4 to position A, valve 3 to HPLP-pump
Switch on the HPLC pump until the pressure is 350 bar
Then, V3 to stop, pump off, valve 4 to B and react 5 minutes (T =)
Switch valve 2 to collection vial (THF solution in collection vial)
Close valve 2, switch V3 to HPLC, valve 4 to A, pump on until the pressure is 350 bar.
Valve 3 to stop, pump off and valve 4 to B
Valve 2 to collection vial
Measure collected activity, flush with 10 mL air, measure again
At the end flush loop and tubing with distilled THF
Default positions: V1 B, V2 Silica, V3 Ascarite, V4 A, HPLC off

Purification:
Load the diluted product fraction (0.5 mL AcN / H2O 1:1) in the syringe (up, load, waste)
Flush with eluent (0.5 mL) once and again load in the syringe
Load the loop (down, load, waste)
Inject on the column (inject)
Fraction collection at app. 9 minutes

Activity balance:
A in collection vial after reaction: **MBq T =**
A after air flush (10 mL): **MBq T =**
A in ascarite: **MBq T =**
A in emptied collection vial: **MBq T =**
A in fraction flacon: **MBq T =**

Methylation protocol:

Date: Hot Cell: Act:

Preparations:
Prepare 3 columns: sicapent, NaOH and sicapent / NaOH
Connect sicapent / NaOH column to distillation tube with long needle for vial 2
Other distillation tube end small needle for vial 1
Porapak column on vial 2
Helium in vial 1 via sicapent column, helium out via NaOH column
Connect HPLC column and eluens
HPLC fraction flacon with exhaust needle
Vial 2: 1 mg precursor, 1 µL TBAOH, 100 µL DMF
Fill 1 mL syringe with 0.22 mL HI solution
Syringe with 2 mL AcN / H2O 1:1, with needle
Hot Cell buttons: Elevator, UV, He

Reaction vial 1: Conversion of CO_2 to CH_3I
Dry reaction vial at 140 °C with 100 mL / minute helium flow
Allow vial to cool to room temperature
Add 0.1 mL 0.1 M $LiAlH_4$ in THF to dried reaction vessel
Harvest CO_2 and stop at the point: Dewar down and compressed air open
Open valve 6 + 7 (gas inlet)
Check for bubbles in the $LiAlH_4$ solution before adding CO_2 (needle down)
Release CO_2 in $LiAlH_4$ solution (Dewar down and compressed air open)
Wait until the activity in the reaction vial is stable
Evaporate THF at 130 °C, 10 mL / minute helium flow (needle up) Valve 6 position a, valve 13 open
When temperature is 130 °C, He-flow 20 seconds at 100 mL / minute (Valve 15)
Adjust helium flow to 10 mL / minute (Valve 15)
Connect distillation tube (sicapent / NaOH) of vial 2 to vial 1
Remove exhaust from vial 1 (NaOH column)
Add 0.22 mL HI solution
Lower needle in vial 2, immediately
Lower needle in vial 1 (helium inlet), immediately (valve 12)
Disconnect He-inlet from sicapent column when activity is in vial 2-> screen unit 2

Reaction vial two:
When activity here is stable remove all needles (distillation tube and Porapak column)
Heat the solution at 80 °C for three minutes
Measure the activity in vial 2 (=A_0)
Dilute reaction mixture with 1 mL AcN / H2O 1:1
Load the solution carefully in the syringe (up, load, waste)
Add 1 mL AcN / H2O 1:1 to reaction vial and load in the syringe again
Load into loop (down, load, waste) and start the run (inject)
C18 alltima, eluent **60:40** acetonitril / water + 0.1 % TFA, retention time appr. 9 minutes
C18 alltima, eluent **55:45** acetonitril / water + 0.1 % TFA, retention time appr. 13 minutes
!Product elutes after precursor peak!

Activity balance:

A reaction vial 2 at end of reaction	**MBq**	**T =**
A in collected fraction	**MBq**	**T =**
A leftover in vial 2	**MBq**	**T =**

Formulation protocol:

Preparations:
Fill flask 1 to 4 on the formulation unit with respectively 50 mL water, 20 mL water, 1 mL ethanol and 10 mL saline with 0.4 % polysorbate (or 2.5 % polysorbate)
Attach fraction tube of HPLC unit to flask 1 containing 50 mL water for injections
Turn on the magnetic stirrer in flask 1
Wash Seppak C18-plus with 5 mL ethanol, followed by 5 mL water and connect in formulation unit
Prepare product flacon with exhaust needle and, if desired, yellow sterile filter with needle

Formulation:
Add prep fraction to flask 1 with 50 mL water and flow through Seppak (WASTE, valve 1 and 5)
Flush Seppak with flask 2, 20 mL water (WASTE, valve 2 and 6)
Switch from WASTE to PRODUCT
1 mL Ethanol through Seppak (valve 7, 6 and 3), then 10 mL saline with 0.4 % polysorbate (valve 7, 6 and 4).

Sterile filtration:
The sterile filter can be immediately connected to the product tube of the formulation unit or the formulated solution can be load a 20 mL Luer lock syringe and connected to a sterile Millipore filter
Empty syringe over the filter in a final product vial

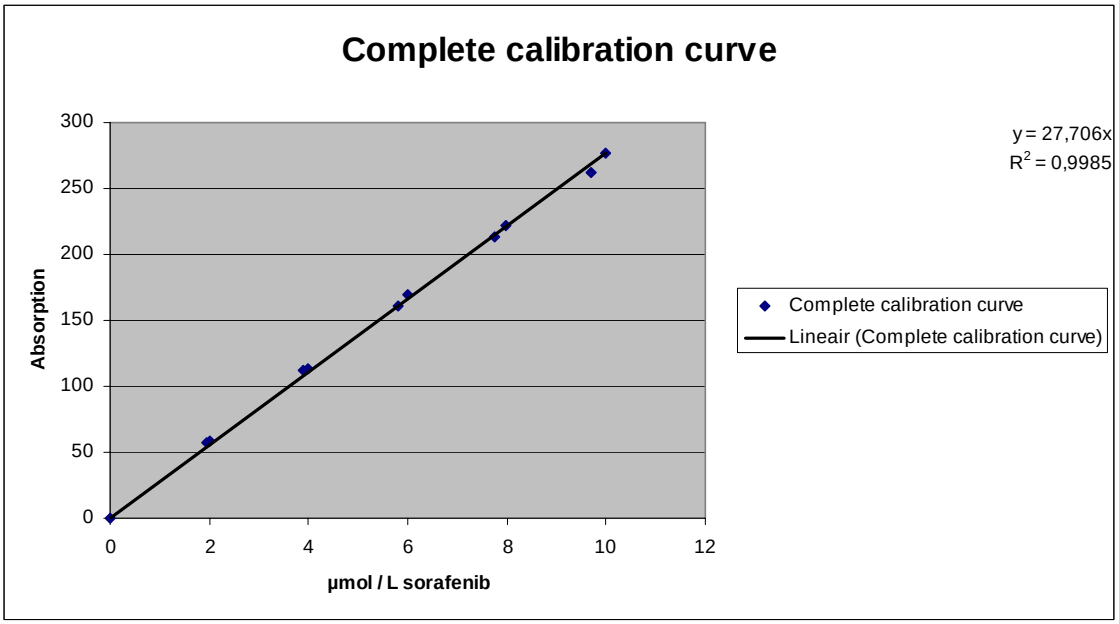
Activity on Seppak column	**MBq**	**T =**
Residual activity in flacon	**MBq**	**T =**
Seppak activity after water flush	**MBq**	**T =**
Eluted product	**MBq**	**T =**
Residual activity on Seppak	**MBq**	**T =**
Activity on filter	**MBq**	**T =**
Activity in flacon	**MBq**	**T =**

Specific activity calibration curve:

In duplo, approximately 1 mg of sorafenib is carefully weighed and dissolved in 5 mL eluent, actonitril / water 1:1 + 0.1 % TFA (stock solution 1 and stock solution 2). From these solutions 100 µL is diluted in 4900 µL eluent (Mother Load). From this solution the other dilutions are made and also this solution will be injected as the highest concentration in the curve. In duplo, four tubes are labelled 1-4 and 200, 400, 600 or 800 µL of the mother load is diluted in these tubes to 1 mL with eluent. Also in duplo, from all these ten solutions, 20 µL is injected on the same HPLC-system (system 4 in C13). The table below depicts the dilution series and the corresponding HPLC-AUC values.

Tube	µL mother load 1	µL mother load 2	µL eluent	µmol / L	AUC-1	AUC-2	Average AUC
1.1	200		800	1,94	57,87	57,11	57,49
1.2	400		600	3,88	113,09	111,02	112,05
1.3	600		400	5,81	162,57	159,41	160,99
1.4	800		200	7,75	217,50	209,25	213,37
ML1	3000		0	9,69	265,00	258,26	261,63
2.1		200	800	2,00	60,47	57,62	59,04
2.2		400	600	3,99	113,41	112,87	113,14
2.3		600	400	5,99	170,27	169,91	170,09
2.4		800	200	7,99	222,72	221,52	222,12
ML2		3000	0	9,98	274,77	279,02	276,89

These AUC values all correspond to a known sorafenib concentration and this way when the AUC value is plotted against the sorafenib concentration this should give a straight line. This line is depicted below.



Now, for a sample with unknown concentration, the AUC can be measured and using this calibration curve (or linear formule y = 27,706 X) the concentration can be determined (or calculated). Specific activity is the amount of GBq / µmol so by measuring the activity of the sample and determining the concentration the specific activity can be determined.