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Synthesis of 2-amino-6-(2-[^{18}F]fluoro-pyridine-4-ylmethoxy)-9-(octyl-β-D-glucosyl)-purine: a novel radioligand for positron emission tomography studies of the O^6-methylguanine-DNA methyltransferase (MGMT) status of tumour tissue

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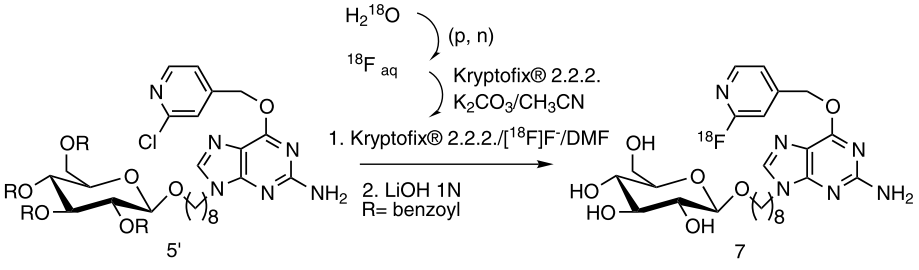
Abstract—The synthesis of the novel glucose conjugated O^6-methylguanine-DNA methyltransferase (MGMT) inhibitor 2-amino-6-(2-[^{18}F]fluoro-pyridine-4-ylmethoxy)-9-(octyl-α-D-glucosyl)-purine is reported. This compound might serve as a radiotracer for the determination of the MGMT status of tumour tissue.

A major disadvantage in cancer therapy is the pre-existing and acquired resistance of tumour cells against chemotherapeutics.[1] The human DNA repair protein O^6-methylguanine-DNA methyltransferase (MGMT) plays a critical role in cancer therapy with alkylating reagents such as alkylnitrosoureas and alkyltriazenes. Tumour cells and neoplastic tissue with elevated MGMT levels can be resistant to alkylating therapeutics. To be able to quantify the MGMT status non-invasively could be helpful in finding the appropriate cancer therapy. Although potent inhibitors of MGMT have been found and introduced into adjuvant therapy, in all cases no selectivity for neoplastic tissue has been achieved and all showed poor water solubility, which limits their applications.[3] Recently, two MGMT-inhibitors were labelled with [^{18}F]fluorine and [^{131}I]iodine, however without glucosidation.[4] Following our first approach to determine the level of MGMT in tumour tissue using ^{18}F-labelled derivatives of O^6-methylguanine,[2] the aim of the present study was to



Scheme 1.

Keywords: positron emission tomography; MGMT; ^{18}F-fluorination.

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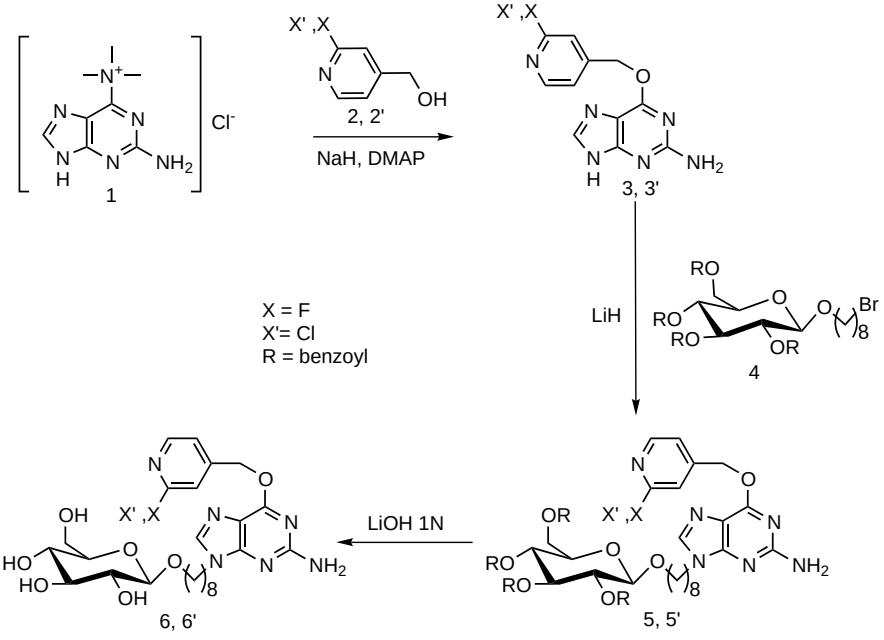
synthesise a new ^{18}F-fluorinated glucose-conjugated MGMT inhibitor for the in vivo determination of the MGMT-status of tumour tissue with positron emission tomography (PET). Glucosidation represents a general strategy for improving both drug solubility and targeting. Thus, appropriate glucosyl derivates of MGMT inhibitors might provide targeted depletion of MGMT activity in neoplastic tissue using the overexpressed glucose-transporters while the uptake in normal tissue is minimised. The labelling procedure employs a novel precursor, chloro-compound **5′**, and dried 2.2.2. Kryptofix®/[^{18}F]fluoride complex in DMF as a solvent. As indicated in Scheme 1, selective nucleophilic aromatic substitution of chloride with [^{18}F]fluoride and subsequent cleavage of the benzoyl-protecting groups gave compound **7** in an overall radiochemical yield of 5% with a specific activity of 80 GBq/μmol at the end of a 60-minute synthesis.

The required precursor **5′** as well as the non-radioactive standard compound **6** were obtained as illustrated in Scheme 2. The O^6-substituted guanine derivatives **3** and **3′** were synthesised from the trimethylammonium salt **1**[11] and the halogeno-pyridine methanols **2**[12] and **2′**[13] in DMSO at 60°C using a new coupling method which applied DMAP as a catalyst.[5] Using this method, compound **3** and **3′** could be obtained in yields of 80–85%. The 8-bromooctyltetra-*O*-benzoyl-β-D-glucopyranoside **4** was obtained as described by Reinhard et al., who reported the synthesis of monosaccharide-linked inhibitors of MGMT in general.[6] Extensive molecular modelling studies revealed that the spacer length of the $(CH_2)_n$ alkyl-linker has to be in the range $n=8$–12 to obtain strongest protein–spacer and protein–glucose interaction. The coupling of **3** or **3′** and **4** to obtain **5** or **5′** was achieved applying the method of Kjellberg and Liljenberg.[7] The use of an equimolar amount of LiH as a base for the deprotonation of the N^9-H-atom and subsequent reaction with **4** in DMF at 80°C within 2–3 h (TLC control) gave only the N^9-substituted product. **5** and **5′** were purified by column chromatography (aceton/*n*-hexane 1:1) to give chemical yields ranging from 20 to 40%. The varying of the chemical yields depend highly on the effectiveness of the column chromatography step. If the flow rate of the mobile phase is too low, deprotection of the benzoyl groups occurs due to the basic nature of the silica gel. Thus, a fast separation of the coupling products via flash chromatography is strongly recommended. Characterisation of **5** and **5′** was accomplished by ^{1}H, ^{13}C, ^{19}F NMR, ESI MS and elemental analysis (Table 1) as well as by gradient reverse-phase HPLC (gradient eluent water/CH_3CN, 100% CH_3CN after 20 min) and normal-phase TLC (aceton/petrolether 4:1). Additionally, the ^{13}C chemical shifts of C-4, C-5 and C-8 of the guanine moieties of **5**, **5′** and **6** were compared with compounds of clearly established structural identity. Chemical shifts were expected to be in the range of 153–155 ppm for C-4, 115–116 ppm for C-5 and 138–140 ppm for C-8.[6] All glucose conjugated compounds displayed ^{13}C chemical shifts within this required range as indicated in Table 1. Furthermore, it is well known that N7-substituted MGMT inhibitors do not show any inhibitory activity at all[6,10] whereas compound **6** which was synthesised from **5** clearly showed MGMT inhibition. No deprotection of the labelling precursor **5′** was performed because the benzoyl groups are a necessary requirement for the radioactive labelling with [^{18}F]fluoride. Every acidic H-atom has to be protected to prevent the formation of a hydrogen–fluorine bond which decreases the nucleophilicity of the [^{18}F]fluoride atom, ultimately leading to lesser radiochemical yields.[9] The final cleavage of the benzoyl protecting groups in the case of **5** could easily be achieved quantitatively by dissolving **5** in methanol and trituration with NaOMe 1N at room temperature for 2 h. Subsequent neutralisation with Dowex (H+ 50WX2) ion exchanger and final



Scheme 2.

Table 1.

	NMR (400 MHz)[a]			ESI MS[b]	Elemental analysis (%)[c]
	^{1}H	^{13}C	^{19}F		
5	δ = 8.2 (d, 1H), 7.9–7.2 (m, 23H), 6.4 (s, 2H), 5.9 (t, 1H), 5.7 (s, 1H), 5.5 (t, 1H), 5.3 (t, 1H), 5.1 (d, 1H), 4.4 (m, 2H), 3.9 (t, 1H), 3.8 (t, 1H), 3.7 (m, 1H), 3.5 (m, 2H), 1.5 (m, 1H), 1.1–0.8 (m, 8H)	δ = 165.6, 165.4, 165.0, 164.8, 162.6, 159.8, 159.7, 155.0, 153.0 (C-4), 148.1, 140.5 (C-8), 136.7, 134.1 134.0, 133.7, 129.6, 129.5, 129.4, 129.3, 129.0, 128.8, 128.7, 120.7, 114.0 (C-5), 108.1, 107.7, 100.1, 73.4, 72.1	δ = −69.1 (d)	885.4 (100) $[M+Na-C_6H_5CO]^+$, 907.5 (5) $[M+2Na-C_6H_5CO]^+$, 923.5 (2) $[M+Na+K-C_6H_5CO]^+$, 989.5 (21) $[M+Na]^+$, 1005.5 (2) $[M+K]^+$	C, 65.21; H, 5.43; N, 9.31

	NMR (400 MHz)[a]		ESI MS[b]	Elemental analysis (%)[c]
	^{1}H	^{13}C		
5′	δ = 8.4 (d, 1H), 7.9 (m, 5H), 7.7 (d, 1H), 7.5 (m, 13H), 6.0 (t, 1H), 5.6 (t, 1H), 5.4 (t, 1H), 5.2 (d, 1H), 4.5 (dd, 2H), 4.0 (dd, 1H), 3.8 (m, 1H), 3.6 (m, 1H), 2.6 (m, 2H), 1.8 (m, 2H), 1.5 (m, 2H), 1.3 (t, 8H)	δ = 165, 163, 160, 159.8, 155 (C-4), 151, 150, 140 (C-8), 134, 129, 123, 122, 114 (C-5), 100, 73, 72, 71, 70, 69, 65, 63, 43, 31, 29, 28, 25.6, 25	983.3 (90) $[M]^+$, 1005.1 (100) $[M+Na]^+$, 861.4 (15) $[M-C_6H_5CO]^+$	C, 64.21; H, 5.61; N, 9.05

	NMR (400 MHz)[a]			ESI MS[b]	Elemental analysis (%)[c]
	^{1}H	^{13}C	^{19}F		
6	δ = 8.2 (d, 1H), 7.7 (s, 1H), 7.4 (d, 1H), 7.2 (s, 1H), 6.4 (s, 2H), 5.7 (s, 2H), 5.5 (s, 2H), 4.9 (m, 4H), 4.8 (d, 1H), 4.4 (t, 1H), 4.0 (m, 2H), 3.9 (m, 2H), 3.7 (m, 1H), 3.6 (m, 1H), 3.0 (m, 2H), 1.7 (m, 3H), 1.4 (m, 4H), 1.2 (m, 4H)	δ = 159, 154.9, 153.0 (C-4), 148.1, 140.6 (C-8), 136.8, 113.8 (C-5), 108.1, 107.7, 103.1, 77.0, 73.7, 70.3, 68.8, 64.8, 61.3, 48.9, 42.8, 29.5, 29.4, 29.0, 28.7, 26.2, 25.7	δ = −69.1 (d)	550.6 (75) $[M]^+$, 468.58 (100) $[M+Na-C_6H_5CO]^+$, 573.6 (46) $[M+Na]^+$	C, 54.87; H, 6.74; N, 15.87

[a] NMRs are recorded in DMSO-d_6.
[b] ESI MS (m/z (% rel. int.)).
[c] Elemental analyses were obtained after preparative gradient HPLC-purification.

workup via column chromatography ($CHCl_3$/MeOH 4:1) gave the pure product **6** which was identified by ^1H, ^{13}C, ^{19}F NMR, ESI MS and elemental analysis (Table 1) as well as by gradient reverse-phase HPLC (gradient eluent water/CH_3CN, 100% CH_3CN after 20 min) and normal-phase TLC ($CHCl_3$/MeOH 4:1).

For the synthesis of radiolabelled compound **7**, a different route was chosen to introduce the [^{18}F]fluoride in one step without performing subsequent synthetic procedures. No-carrier-added (NCA) aqueous [^{18}F]fluoride prepared by the $^{18}O(p,n)^{18}F$ nuclear reaction on an enriched water target (95+% ^{18}O) was added to a solution of K_2CO_3 1N (15 μl)/Kryptofix® 2.2.2. (10 mg) in a Pyrex vessel. The water was evaporated using a stream of nitrogen at 80°C and co-evaporated to dryness with CH_3CN (2×1 ml). Approximately 90% of the starting [^{18}F]F-radioactivity could be recovered after the drying procedure. Precursor **6′** (10 mg, 0.01 mmol) in 0.4 ml DMF was added to the dried K[^{18}F]F/Kryptofix® 2.2.2. complex and the solution was heated at 100°C for 15 min. The radiochemical yield for this substitution of the chlorine atom by the [^{18}F]fluorine isotope is approximately 15%. This relatively low yield could possibly be explained by assuming a sterical hindrance of the chlorine-[^{18}F]fluorine substitution via the bulky glucose unit which is freely movable due to the long chain alkyl linker. LiOH 1N (0.1 ml) was added and heating was continued for 10 min leading to a quantitative deprotection of the glucose unit. The resulting mixture was cooled to rt, Dowex (H+ 50WX2) ion exchanger (70 mg) was added and stirred for an additional 3 min to ensure the complete protonation of the glucose moiety. The solution was passed through a PTFE-filter (1 μm) and transferred to an HPLC-system. The radioactive product corresponding to **7** was isolated by gradient reversed-phase HPLC (gradient eluent water/CH_3CN, 100% CH_3CN after 20 min) in high chemical and radiochemical purity (>99%). The synthesis and purification of the radioligand for biological investigations were accomplished within 60 min from end of bombardment of the target and gave compound **7** in an average overall radiochemical yield of 5%. This procedure is thus compatible with the short half-life (110 min) of fluorine-18 and will facilitate tomographic studies of MGMT protein status in normal and disease tissues.

In addition, for proving the feasibility of the non-radioactive standard compound, a MGMT assay of compound **6** was performed to obtain the IC_{50} value which provides important information about the quality of the correspondent ^{18}F-labelled radiotracer. The MGMT assay has been previously described in detail.[8] For compound **6**, an IC_{50} value of 1.2 μM was determined which seems to be high enough for PET.

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