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Potent blockers of the monocarboxylate transporter MCT1: Novel immunomodulatory compounds

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Abstract—A novel series of potent blockers of the monocarboxylate transporter, MCT1, is disclosed. From very potent but lipophilic lead compounds, systematic changes to all parts of the molecule, targeting reduction in log *D*, afforded compounds with significantly improved overall properties. These compounds show potent immunomodulatory activity.

An important component of the immune response is activation of T-cells following antigen challenge. However, undesirable activation can lead to graft rejection following transplantation and to autoimmune diseases such as rheumatoid arthritis.

Upon activation, T-cells produce the autocrine growth factor, IL-2, whose regulation relies on the nuclear factor of activated T-cells (NFAT-1).[1] Tacrolimus™ (FK-506) and cyclosporin, when bound to their immunophilin (FKBP and cyclophilin, respectively), reduce IL-2 production by inhibiting calcineurin, which in turn reduces NFAT-1 translocation to the nucleus.[2] While both compounds are effective clinical agents, renal and other toxicities limit their widespread use. Researchers at Sterling Winthrop Pharmaceuticals pursued an alternative, calcineurin independent, approach to IL-2 inhibition via NFAT-1 and identified a number of interesting compounds including the pyrrolopyrimidinedione **1**.[3]

Intrigued by these results, we chose to investigate this area further. The synthesis and properties of a novel series of compounds derived from **1** are presented below. Importantly, we demonstrated that in primary T-lymphocytes these compounds do not exert their activity through effects on IL-2 regulation. Moreover, through compound-led target identification[4] we were able to show, by photoaffinity labelling and proteomic characterisation, that the actual molecular target of these compounds is the monocarboxylate transporter, MCT1. This was supported by a strong correlation between binding at MCT1 and in vitro immunomodulatory activity in an assay measuring inhibition of PMA/ionomycin-induced human PBMC proliferation.[5]

The monocarboxylate transporters are a family of proteins which transport lactate and other small monocarboxylates. We have shown that MCT1 expression is rapidly upregulated upon T-lymphocyte activation in order to meet the demand for lactate efflux resulting from an increased glycolytic rate. Inhibition of lactate efflux by potent blockade of lactate transport results in the accumulation of lactate within the cell and feedback inhibition of glycolysis. This suppression of cellular metabolism results in the inability of T-lymphocytes to sustain the rapid rate of cell division occurring during the early immune response to antigen recognition, without being cytotoxic. Blockade of MCT1 is thus a

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novel mechanism of immunosuppression distinct from current therapies.

Initial chemical effort focussed on synthesising compounds with changes to the heterocyclic scaffold. Amongst numerous new cores investigated, those contained in compounds **2** and **3**, prepared as previously described, were most interesting (Fig. 1).[5] The new compounds displayed excellent affinity for MCT1. However, these compounds were too lipophilic and, as a consequence, suffered from poor aqueous solubility and high in vitro metabolic clearance in human hepatocytes as well as potent CYP2C9 inhibition (Table 1).

In modern drug discovery programmes, it is well recognised that compound optimisation often results in an increase in both molecular complexity and lipophilicity, driven by the need to increase potency, thereby defining the required lead properties and resulting in the concept of 'leadlikeness'.[6,7] Given the high starting potency in this case, our strategy, conversely, was to substantially reduce lipophilicity while accepting some reduction in activity in order to improve overall properties.

To explore 5- and 6-substituent changes (see Fig. 1 for numbering), the pyrimidinothiophene ring system was used as this facilitated introduction of diversity at both positions. Initially 2-trifluoromethylphenylmethyl was chosen as the 6-position (e.g., **4**), as this gave similar properties to naphthylmethyl (e.g., **3**). The 5,6-unsubstituted pyrimidinothiophene **5**[5] was selectively brominated at the 6-position. Following deprotonation with LDA at the 5-position, a bromide migration occurred.[8] The resulting anion **6** was reacted with 2-trifluoromethylbenzaldehyde to provide the 5-bromo intermediate **7**. Oxidation of this compound gave the keto compound **8** which was now activated towards nucleophilic attack at the 5-position. Following addition of the desired thiol the keto group could be removed via a standard two-step process (Scheme 1). Alternatively, deoxygenation of bromothiophene **7** gave the key intermediate **9** having the desired 6-substituent and a functionalised 5-position which, following halogen–metal exchange, could be reacted with a range of electrophiles. Reaction with sulfur provided a route to functionalised thioethers (Scheme 2) while quenching with sulfur dioxide or carbon dioxide allowed the synthesis of sulfonamides and amides (Scheme 3).

The homologous thioethers **10**–**14** showed significantly improved in vitro metabolic stability over **4** and had comparable affinities for MCT1, albeit less than that of the starting compound. Reintroducing hydroxyl groups on the cyclopentyl thioether gave a range of compounds **15**–**18** which generally displayed good affinity but also showed high intrinsic clearance in human hepatocytes. The dihydroxypropyl derivative **19** was significantly less active than the monohydroxypropyl lead **4** and showed no improvement in hepatic clearance (Table 2).

These results indicated that, although the hydroxy group interacted favourably with the transporter giving high activity, it was detrimental to metabolic stability. In agreement with this, examination of the metabolite profile following incubation of compound **3** in human hepatocytes indicated that the primary route of metabolism was glucuronidation on the hydroxyl group.

Although reducing lipophilicity was an ultimate goal of this work, in this initial phase it can be seen that measured log *D* values remained high for all compounds, within a range much narrower than calculated, and displaying variations which did not follow the expected trend (see measured vs calculated values, Table 2). For example, addition of a second hydroxyl group in going from **4** to **19** led to an increase in log *D* where a reduction would be expected. Similarly, in the non-hydroxylated compounds, sequentially increasing the number of carbons from ethyl **10** to cyclohexyl **14** reduced log *D* where a significant increase would be anticipated. It is likely that this inconsistency is due to 'hydrophobic collapse,' that is, lipophilic 5- and 6-substituents interact intramolecularly leading to less than expected bulk lipophilicity.[9]

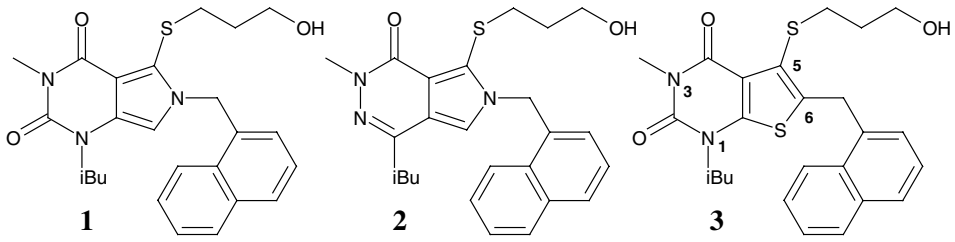
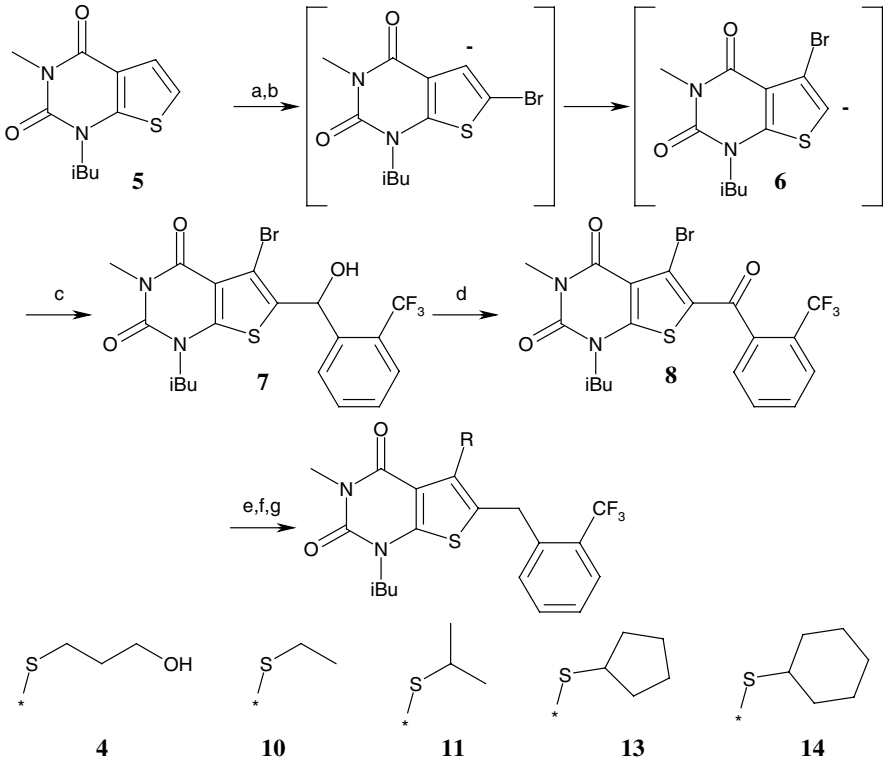


Figure 1. Structures of compounds **1**–**3**.

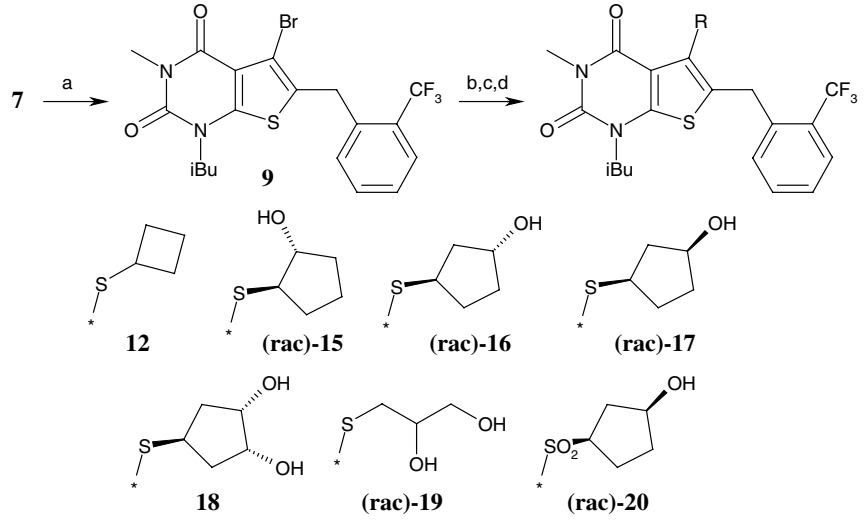
Table 1. Comparison of heterocyclic scaffolds

Compound	MCT1 binding K_i[a] (nM)	Human Heps Cl_{int} (μL/min/10^6cells)	CYP2C9 IC_{50} (μM)	Sol (mg/mL)	log *D*
1	0.33	nd	nd	0.08	4.4
2	0.10	36	2.60	<0.001	4.8
3	0.28	58	0.50	<0.001	4.8

nd, not determined.
[a] FB binding (Ref. 5).



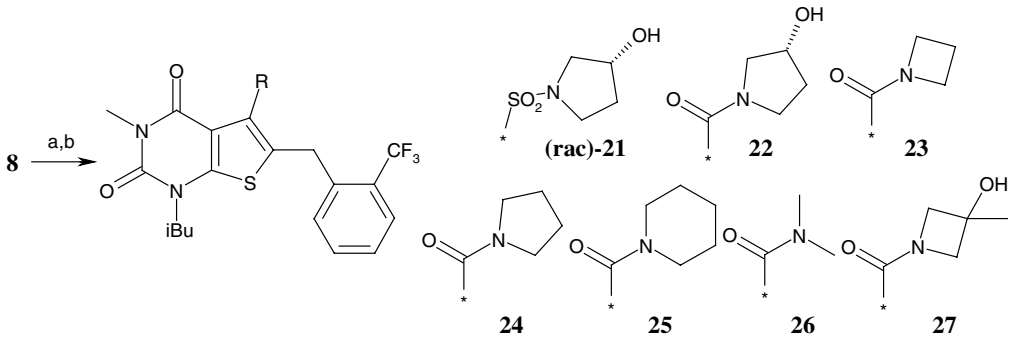
Scheme 1. Reagents and condition: (a) Br_2, DCM, 98%; (b) LDA, THF, −78 °C; (c) 2-$CF_3C_6H_4CHO$, 71% (two steps); (d) tetrapropylammonium perruthenate, NMO, 4 Å molecular sieves, DCM, 97%; (e) RSH, K_2CO_3, DMF, 65–97%; (f) $NaBH_4$, MeOH, 77–90%; (g) TFA, Et_3SiH, 48–93%.



Scheme 2. Reagents and condition: (a) TFA, Et_3SiH, 83%; (b) *i*-PrMgCl, THF, 0 °C; (c) S_8; (d) for **12**: bromocyclobutane, $NaBH_4$, $NaHCO_3$, H_2O, THF, 36% (three steps); for (***rac***)-**15**: cyclopentane epoxide, $NaBH_4$, $NaHCO_3$, H_2O, THF, 36% (three steps); for (***rac***)-**16** and (***rac***)-**17**: i—2-cyclopentenone, dil HCl(aq), THF; ii—$NaBH_4$, MeOH, 4% and 16% (four steps); for **18**: i—3-tosyloxycyclopentene, $NaHCO_3$, H_2O, THF; ii—OsO_4, NMO, THF, H_2O, 7% (four steps); for (***rac***)-**19**: glycidol, $NaBH_4$, $NaHCO_3$, H_2O, THF, 23% (three steps); for (***rac***)-**20**: *m*CPBA, DCM (on (***rac***)-**17**), 10% (four steps).

In an effort to significantly reduce measured lipophilicity, changes to the thioether link were made, exemplified here for the hydroxycyclopentyl series which ultimately provided one of the most favoured 5-substituents. Replacing thioether **17** with sulfone **20** gave a drop in activity and very high CYP2C9 inhibition. Replacing the cyclopentane with the equivalent pyrrolidine to give the sulfonamide **21** improved activity, however, CYP2C9 inhibition was again high. The analogous amide **22** had the lowest log *D* in this set, displayed good affinity and also showed improved human hepatocyte stability and lower CYP2C9 inhibition relative to the sulfone and sulfonamide analogues (Table 3).

More amide analogues were also evaluated (Table 4). In this series, the non-hydroxylated 4, 5 and 6-membered



Scheme 3. Reagents and conditions: (a) *i*-PrMgCl, THF, 0 °C; (b) for **21**: i—SO_2; ii—NCS, HCl(aq), DCM; iii—(*R*)-3-hydroxypyrrolidine, triethylamine, DCM, 70% (four steps); for **22–27**: i—CO_2, 0 °C, 75% (two steps); ii—oxalyl chloride, cat. DMF, DCM; iii—RR′NH·HCl, triethylamine, DCM, 22–67% (two steps).

Table 2. Comparison of 5-substituents

Compound	MCT1 binding K_i[a] (nM)	Human Heps Cl_{int} (μL/min/10^6 cells)	log *D*	Calculated log *D*[b]
4	0.35	43	4.1	5.7
10	6.0	nd	4.5	6.6
11	2.2	2	4.4	6.9
12	13	3	>3.5	7.0
13	3.2	4	4.2	7.5
14	6.0	4	4.1	8.1
15	2.7	11	4.2	5.8
16	0.29	26	nd	5.7
17	0.42	31	4.4	5.7
18	0.68	82	4.4	4.7
19	6.5	59	>4.5	4.7

nd, not determined.
[a] FB binding (Ref 5).
[b] Ref. 10.

Table 3. Comparison of 5-linking group

Compound	MCT1 binding K_i[a] (nM)	Human Heps Cl_{int} (μL/min/10^6 cells)	CYP2C9 IC_{50} (μM)	log *D*
17	0.42	31	0.2	4.4
20	12	31	0.2	3.2
21	1.1	82	0.2	3.3
22	4.9	17	1.7	2.7

[a] FB binding (Ref. 5).

Table 4. Comparison of 5-amides

Compound	MCT1 binding K_i[a](nM)	Human Heps Cl_{int} (μL/min/10^6 cells)	log *D*
22	4.9	17	1.7
23	9.2	18	3.5
24	5.5	27	3.8
25	8.7	nd	4.1
26	37	11	2.9
27	3.5	7	3.2

nd, not determined.
[a] FB binding (Ref. 5).

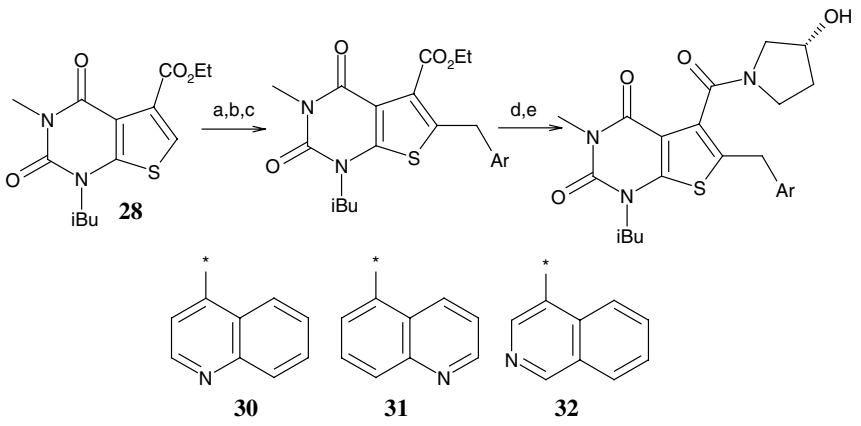
rings (**23**, **24** and **25**) showed similar properties, while the smaller dimethyl amide **26** was less active. In this case, the log *D* trend was in the expected direction. The hydroxy-substituted azetidine **27** showed potency similar to that of the non-hydroxy derivative **23** but was more metabolically stable, perhaps due to the alcohol being tertiary.

Alternative groups at both the 1- and 3-positions were also examined but no substituents significantly better than the original methyl and isobutyl were found (data not shown).

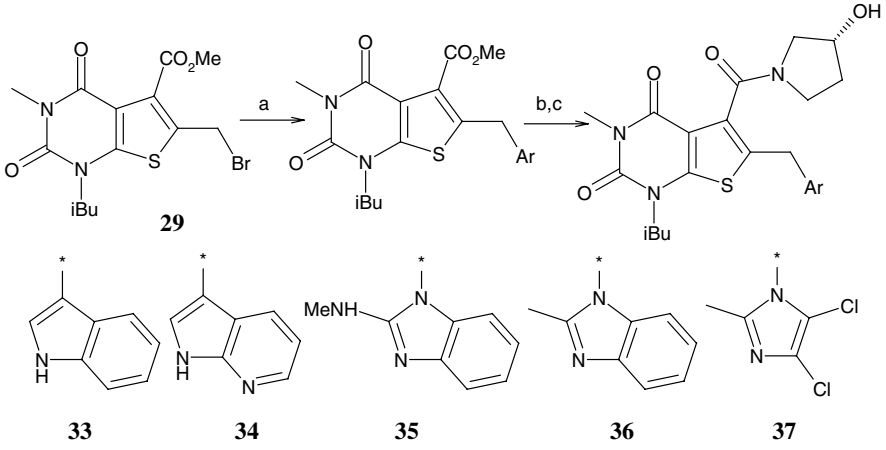
Attention was then turned to the 6-substituent. In order to prepare a range of 6-heterocyclic 5-amides, predicted to have lower log *D*, the synthesis was modified to allow incorporation of the 6-substituent at a later stage via key intermediates **28** or **29** (Schemes 4 and 5).[5]

While a range of 6-heteroaryl compounds were prepared with various different 5-amides, results are again presented here for the (*R*)-5-hydroxypyrrolidine amide series derived from **22** as this was found to be amongst the best 5-amides and the trends described are representative of those found with other amides (Table 5). The quinolines **30** and **31** gave good potency and stability along with an appreciably lower log *D* relative to the trifluoromethylphenyl derivative **22**, **30** being the better isomer with respect to CYP2C9 inhibition. 4-Isoquinoline **32** showed very potent CYP3A4 inhibition. The indole **33** had properties similar to those of **22** but suffered from high inhibition of both CYP2C9 and CYP3A4. The azaindole **34** was more stable, had much reduced CYP inhibition and had a lower log *D* than the indole **33** but was also less active. In the imidazole series, the 2-methylamino benzimidazole **35** gave the best balance of properties with good potency, very good stability, low CYP inhibition and low log *D*. The methyl benzimidazole **36** and methyl imidazole **37** were both less active. It is noteworthy that the combination of an amide at the 5-position and a heteroaryl group at the 6-position was required in order to reduce the log *D* to the desired range.

In summary, novel MCT1 blockers have been discovered which show potent inhibition of PMA/ionomycin-induced human PBMC proliferation. By reducing log *D*, the drug-like properties of the series were significantly improved with respect to solubility, plasma protein binding (ppb), human metabolic stability and CYP2C9 inhibition (cf. **3** and **30**, Table 6). Good potency was



Scheme 4. Reagents and condition: (a) ArCHO, LDA, THF, −78 °C, 56–84%; (b) Ac_2O, TEA, DMAP, DCM or $MeSO_2Cl$, DMAP, TEA, THF; (c) H_2, Pd/C, $NaHCO_3$, H_2O, MeOH, 28–78% (two steps); (d) NaOH, H_2O, THF, MeOH, 79–88%; (e) RR′NH·HCl, EDCI, HOBt, DCM or PyBrOP, DIPEA, THF, 62–97%.



Scheme 5. Reagents: (a) indole, $NaHCO_3$, H_2O, $CHCl_3$, 63% or azaindole, NaH, $ZnCl_2$, toluene, 55% or 'imidazole,' NaH, DMF, 11–82%; (b) NaOH, H_2O, THF, MeOH; (c) RR′NH·HCl, EDCI, HOBt, DCM or PyBrOP, DIPEA, THF, 7–58%.

Table 5. 6-Substituent changes

Compound	MCT1 binding K_i[a] (nM)	Human Heps Cl_{int} (μL/min/10^6 cells)	CYP2C9 IC_{50} (μM)	CYP3A4 IC_{50} (μM)	log *D*
22	1.9	17	1.7	nd	2.7
30	4.8	6	16	16	1.7
31	5.3	<3	6.0	20	2.0
32	3.9	nd	13	<0.07	1.7
33	0.79	6	1.4	1.0	2.4
34	4.7	<3	35	6.7	1.7
35	6.6	<3	11	>50	1.6
36	16	<3	2.5	>50	1.3
37	26	<3	8.7	>50	1.6

nd, not determined.
[a] SPA binding (Ref. 11).

Table 6. Summary of progress

Compound	MCT1 binding K_i[a] (nM)	Human Heps Cl_{int} (μL/min/10^6 cells)	CYP2C9 IC_{50} (μM)	Sol (mg/mL)	log *D*	ppb (% free)
3	0.08	58	0.50	<0.001	4.8	0.1
30	4.8	6	16	0.52	1.7	36

[a] SPA binding (Ref. 11).

achieved due to the very high potency of the lead compound. Unfortunately, many of the amides described here, including **30**, exist as a mixture of conformational isomers resulting from restricted rotation about both the amide carbon–nitrogen and the carbonyl–aryl bonds. Further studies with these compounds, including atropisomeric properties and implications, will be reported in due course.

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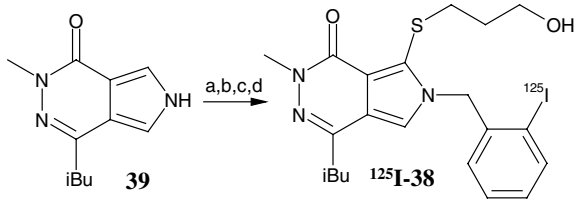
10. ACDlogD is available from ACD Labs, Toronto, Canada, <http://www.acdlabs.com>.
11. A radioligand-binding assay was developed using scintillation proximity assay (SPA) technology (Bosworth, N.; Towers, P. *Nature* **1989** *341*, 167) Wheatgerm agglutinin SPA beads (Amersham) (0.2 mg/mL) coated with Jurkat cell membranes were incubated with [^{125}I]-**38** (0.1 nM) in the absence or presence of increasing concentrations of test compound. Unlabelled **3** (1 μM) was used to define non-specific binding. The test compounds were diluted in DMSO and then assay buffer (50 mM HEPES, 0.1 mM EDTA and 150 mM NaCl, pH 7.4) containing 0.5% bovine serum albumin (BSA) such that the final concentration of DMSO in the assay was 0.5%. The final concentration of BSA in the total assay volume of 200 μL was 0.05%. The assay reagents were incubated for 18 h at room temperature and radioactivity bound to the membrane-coated beads was determined using a Topcount scintillation counter (Perkin Elmer).

The radioligand ^{125}I-**38** was prepared from the heterocycle **39** (Bantick, J.; Cooper, M.; Perry, M.; Thorne, P. WO9929695) as shown below.



(a) i—2-chloromethyliodobenzene, Cs_2CO_3, DMF; ii—LDA, $TsS(CH_2)_3OTBDMS$, THF; (b) $(CH_3)_6Sn_2$, $Pd(PPh_3)_4$, toluene; (c) chloramine-T, $Na^{125}I$, methanol; (d) TBAF, THF.