A Method for Parallel Solid Phase Synthesis of Iodinated Analogues of the CB₁ Receptor Inverse Agonist Rimonabant

Alan C. Spivey,^{*,†} Chih-Chung Tseng,[†] Teyrnon C. Jones,[†] Andrew D. Kohler,[‡] George J. Ellames[‡]

[†]Department of Chemistry, Imperial College, London, SW7 2AY, UK and [‡]Department of Isotope Chemistry and Metabolite Synthesis, sanofi-aventis, Willowburn Ave., Alnwick, Northumberland, NE66 2JH, UK.

a.c.spivey@imperial.ac.uk

Received Date (will be automatically inserted after manuscript is accepted)

ABSTRACT

A method for the parallel solid phase synthesis (SPS) of iodinated analogues of sanofi-aventis' type 1 cannabinoid (CB₁) receptor inverse agonist rimonabant (acomplia[®]) has been developed. The method allows the synthesis of a range of C3 amide/hydrazide derivatives from a resin-bound C3 ester precursor. The C-Ge linkage to the Hypogel[®]-200 resin is stable to the diversification conditions but allows *ipso*-iododegermylative cleavage using NaI/NCS even for the products containing the oxidatively labile hydrazide moiety.

Positron emission tomography (PET) and single photon emission computerized tomography (SPECT) are non-invasive, high resolution 'molecular' imaging techniques used clinically for diagnosis and monitoring of many medical disorders.¹ The short-lived positron or gamma-ray emitting nuclei required for these techniques [*e.g.* ¹²⁴I (*t*_{1/2} 4.2 d) and ¹²³I (*t*_{1/2} 13.2 h), respectively] must be incorporated into the appropriate imaging molecules regioselectively and rapidly.² For radio-iodinated aryl iodides this normally entails *ipso*-iododestannylation of

an aryl trialkyltin precursor (*e.g.* ArSnMe₃ or ArSn^{*n*}Bu₃) by radiolabelled KI or NaI in the presence of an oxidant followed by HPLC purification to remove all traces of toxic tin residues from the product.³

We envisaged that by employing an aryl labelling precursor bound to an insoluble polymer *via* a trialkylgermane linker it would be possible to effect analogous *ipso*-iododegermylative labelling thereby obviating the need for the radiochemist to handle tin-containing materials and also the requirement for any post reaction purification.^{4,5} Ge-containing compounds do not

¹ (a) Ametamey, S. M.; Honer, M.; Schubijer, P. A. *Chem. Rev.* **2008**, *108*, 1501, (b) Willmann, J. K.; van Bruggen, N.; Dinkelborg, L. M.; Gambhir, S. S. *Nat. Rev. Drug Disc.* **2008**, *7*, 591.

² For an excellent overview of radiolabelling see: (a) Miller, P. W.; Long, N. J.; Vilar, R.; Gee, A. D. *Angew. Chem. Int. Ed.* **2008**, *47*, 8998. For radioiodination see: (b) Finn, R. *Chemistry Applied to Iodine Radionuclides in Handbook of Radiopharmaceuticals: Radiochemistry and Applications*, Welch, M. J.; Redvanly C. S. (Eds), John Wiley and Sons, **2003**, (c) Bourdoiseau, M., *Int. J. Radiat. Applic. Instrument. Part B. Nucl. Med. Biol.* **1986**, *13*, 83.

³ (a) Kabalka, G. W.; Varma, R. S. *Tetrahedron* **1989**, *45*, 6601, (b) Glaser, M.; Luthra, S. K.; Brady, F. *Int. J. Oncol.* **2003**, *22*, 253.

⁴ For pioneering studies on the use of arylgermanes for (radio)halogenation see: (a) Moerlein, S. M. *J. Chem. Soc., Perkin Trans. 1*, **1985**, 1687, (b) Moerlein, S. M. *J. Org. Chem.* **1987**, *52*, 664. For the use of polymer-supported arylstannanes for radioiodination see: (c) Culbert, P.; Hunter, D. H. *Reactive Polymers* **1993**, *19*, 247, (d) Hunter, D. H.; Zhu, X. J. *Labelled Compd. Radiopharm.* **1999**, *42*, 653.

have the adverse toxicity profile of their Sn congeners⁶ and the only molecules released into solution should be those of the labelled product. Moreover, unlike arylstannanes, arylgermanes are robust towards a wide range of conditions for chemical synthesis⁷ and so afford the possibility to make a range of derivatives of an advanced precursor prior to final radioiodinative release; a potentially valuable feature during preclinical imaging agent development. Herein, we describe proof-of-concept for this approach as exemplified by the parallel, solid phase synthesis (SPS) of a small array of iodinated analogues of the CB₁ receptor inverse agonist rimonabant.

Type 1 cannabinoid (CB₁) receptors belong to the rhodopsin family of G protein coupled receptors (GPCRs) and are widely distributed in the central nervous system (CNS).⁸ Physiologically, they are implicated in regulation of pain, energy distribution and metabolism. Drug candidates acting as antagonists/inverse agonists for these receptors have been/are in clinical trials for treatment of obesity, metabolic syndrome and various addictions including smoking.⁹ Rimonabant (**1a**, marketed as acomplia®) is a CB₁ receptor inverse agonist first approved for treatment of obesity by the European Medicines Agency in 2006 and then withdrawn from the market in 2008 following the emergence of psychiatric disorders among patients.¹⁰ Various ¹¹C-labelled,¹¹ ¹⁸F-labelled,¹² and I^{123/124}-labelled¹³ derivatives (e.g. **1b**

[¹²³I]AM251 and **1c** [¹²³I]AM281, Fig. 1) have proved to be viable for PET and SPECT imaging of CNS activity but an optimal ligand has yet to be identified.¹⁴

Figure 1 Structures of rimonabant, AM251 & AM281

All reported rimonabant-derived radioiodinated ligands contain the labelled iodine atom in place of the chlorine atom in the C5 aryl ring; this substitution marginally reduces affinity but increases selectivity for binding at CB₁ vs. CB₂. Their synthesis involves solution phase Pd(0)-mediated stannylation of an arylbromide late intermediate (e.g. **5** → **6**, Scheme 1) then *ipso*-iododestannylation with a radiolabelled iodonium source (e.g. **6** → **1c**, Scheme 1).¹³

Scheme 1 Standard synthesis of a radio-iodinated rimonabant analogue (e.g. AM281)

Kabalka, G.F.; Namboodiri, V. Akula, M. *J. Labelled Compd. Radiopharm.* **2001**, *44*, 921.

⁵ (a) For a review of solid phase radiochemistry see: Hockley, B. G.; Scott, P. J. H.; Kilbourn, M. R. in *Linker Strategies in Solid-Phase Organic Synthesis*, Ed. Scott, P. J. H. Wiley, Chichester, 2009, Ch. 22, (b) For an overview of isotopic labelling employing phase-tagged Ge linkers see: Spivey, A. C.; Martin, L. J.; Noban, C.; Jones, T. C.; Ellames, G. J.; Kohler, A. D. *J. Label. Compd. Radiopharm.* **2007**, *50*, 281.

⁶ Lukevics, E.; Pudova, O. In *Chemistry of Organic Germanium, Tin and Lead Compounds*; Rappoport, Z., Ed.; Wiley: Chichester, **2002**; Vol. 2(Part2), p 1685.

⁷ Spivey, A. C.; Tseng, C.-C.; Hannah, J. P.; Gripton, C. J. G.; Fraire de, P.; Parr, N. J.; Scicinski, J. *J. Chem. Commun.* **2007**, 2926 and references therein.

⁸ Howlett, A. C.; Barth, F.; Bonner, T. I.; Cabral, G.; Casellas, P.; Devane, W. A.; Felder, C. C.; Herkenham, M.; Mackie, K.; Martin, B. R.; Mechoulam, R.; Pertwee, R. G. *Pharmacol. Rev.* **2002**, *54*, 161.

⁹ (a) Jagerovic, N.; Fernandez-Fernandez, C.; Goya, P. *Curr. Top. Med. Chem.* **2008**, *8*, 205, (b) Sviženská, I.; Dubový, P.; Šulcová, A. *Pharmacol. Biochem. Behavior* **2008**, *90*, 501, (c) Cota, D. *Diabetes Metab. Res. Rev.* **2007**, *23*, 507, (d) Mucciolo, G. G. *Chem. Biodivers.* **2007**, *4*, 1805, (e) Pagotto, U.; Marsicano, G.; Cota, D.; Lutz, B.; Pasquali, R. *Endocrine Rev.* **2006**, *27*, 73, (f) Ramos, J. A.; González, S.; Sagredo, O.; Gómez-Ruiz, M.; Fernández-Ruiz, J. *Mini-Rev. Med. Chem.* **2005**, *5*, 609.

¹⁰ Jones, D. *Nature Rev. Drug Disc.* **2008**, *7*, 961.

¹¹ Mathews, W. B.; Scheffel, U.; Rauseo, P. A.; Ravert, H. T.; Frank, R. A.; Ellames, G. J.; Herbert, J. M.; Barth, F.; Rinaldi-Carmona, M.; Dannals, R. F. *Nucl. Med. Biol.* **2002**, *29*, 671.

¹² (a) Liu, P.; Lin, L. S.; Hamill, T. G.; Jewell, J. P.; Lanza, Jr, T. J.; Gibson, R. E.; Krause, S. M.; Ryan, C.; Eng, W.; Sanabria, S.; Tong, X.; Wang, J.; Levorse, D. A.; Owens, K. A.; Fong, T. M.; Shen, C.-P.; Lao, J.; Kumar, S.; Yin, W.; Payack, J. F.; Springfield, S. A.; Hargreaves, R.; Burns, H. D.; Goulet, M. T.; Hagmann, W. K. *J. Med. Chem.* **2007**, *50*, 3427, (b) Burns, H. D.; Van Laere, K.; Sanabria-Bohórquez, S.; Hamill, T. G.; Bormans, G.; Eng, W.; Gibson, R.; Ryan, C.; Connolly, B.; Patel, S.; Krause, S.; Vanko, A.; Van Hecken, A.; Dupont, P.; De Lepeleire, I.; Rothenberg, P.; Stoch, S. A.; Cote, J.; Hagmann, W. K.; Jewell, J. P.; Lin, L. S.; Liu, P.; Goulet, M. T.; Gottesdiener, K.; Wagner, J. A.; de Hoon, J.; Mortelmans, L. Fong, T. M.; Hargreaves, R. *J. Proc. Natl. Acad. Sci. USA*, **2007**, *104*, 9800, (c) Li, Z.; Gifford, A.; Liu, Q.; Thotapally, R.; Ding, Y.-S.; Makriyannis, A.; Gatley, S. J. *Nucl. Med. Biol.* **2005**, *32*, 361, (d) Willis, P. G.; Pavlova, O. A.; Chefer, S. I.; Vaupel, D. B.; Mukhin, A. G.; Horti, A. G. *J. Med. Chem.* **2005**, *48*,

5813, (e) Mathews, W. B.; Scheffel, U.; Finley, P.; Ravert, H. T.; Frank, R. A.; Rinaldi-Carmona, M.; Barth, F.; Dannals, R. F. *Nucl. Med. Biol.* **2000**, *27*, 757.

¹³ (a) Berding, G.; Schneider, U.; Gielow, P.; Buchert, R.; Donnerstag, F.; Brandau, W.; Knapp, W. H.; Emrich, H. M.; Müller-Vahl, K. *Psych. Res.: Neuroimaging* **2006**, *147*, 249, (b) Gielow, P.; Klinge, P.; Knapp, W. H.; Berding, G. *Appl. Rad. Isotopes* **2006**, *64*, 763, (c) Berding, G.; Müller-Vahl, K.; Schneider, U.; Gielow, P.; Fitschen, J.; Stuhmann, M.; Harke, H.; Buchert, R.; Donnerstag, F.; Hofmann, M.; Knoop, B. O.; Brooks, D. J.; Emric, H. M.; Knapp, W. H. *Biol. Psychiatry* **2004**, *55*, 904, (d) Lan, R.; Lu, Q.; Fan, P.; Gatley, J.; Volkow, N. D.; Fernando, S. R.; Pertwee, R.; Makriyannis, A. *AAPS Pharmsci* **1999**, *1*, article 4, (e) Gatley, S. J.; Lan, R.; Volkow, N. D.; Pappas, N.; King, P.; Wong, C. T.; Gifford, A. N.; Pyatt, B.; Dewey, S. L.; Makriyannis, A. *J. Neurochem.* **1998**, *70*, 417, (f) Lan, R.; Gatley, S. J.; Makriyannis, A. *J. Labelled. Compd. Radiopharm.* **1996**, *38*, 875, (g) Gatley, S. J.; Gifford, A. N.; Volkow, N. D.; Lan, R.; Makriyannis, A. *Eur. J. Pharmacol.* **1996**, *307*, 331.

¹⁴ For reviews see: (a) Van Laere, K. *Eur. J. Nucl. Med. Mol. Imaging* **2007**, *34*, 1719, (b) Gifford, A. N.; Makriyannis, A.; Volkow, N. D.; Gatley, S. J. *Chem. Phys. Lipids* **2002**, *12*, 65.

Experimental structure activity relationship (SAR) data, particularly in the context of previous attempts to develop effective PET ligands, show that the nature of the C3 hydrazide moiety has a profound effect on the affinity and biodistribution of rimonabant analogues for the CB₁ receptor,^{9a, 15} making this unit an attractive target for variation to allow imaging agent optimization. Consequently, our plan was to prepare a 1,5-diarylpyrazole bound to an insoluble polymer support by a Ge linker (attached at the *para* position of the C5 aryl ring) and containing an ester at C3 that could be readily converted *via* parallel SPS into an array of hydrazide/amide derivatives. Such a strategy would allow the rapid preparation of an array of potential imaging agents from a single functionalized polymer *provided* that the Ge-linker was stable to the C3 diversification protocol and *ipso*-iododestannylation could be achieved in the presence of an oxidatively labile¹⁶ hydrazide moiety. Arylgermanes are uniquely stable towards basic and nucleophilic conditions¹⁷ as compared to arylstannane and arylsilane congeners¹⁷ so we were confident of the former requirement but we were cognizant that arylgermanes undergo S_EAr reactions less rapidly than arylstannanes (due to their lesser β-effect)¹⁸ and so we expected careful optimization of the cleavage conditions would be required to allow rapid clean cleavage without side-reactions (Scheme 2).

Scheme 2 Planned approach to an array of iodinated rimonabant analogues **1** *via ipso*-iododestannylation of functionalised polymers prepared by parallel SPS.

Our initial studies used a solution phase model system **11** which was prepared by transmetalation of lithiated 1,5-diarylpyrazole *tert*-butyl ester **4** (Scheme 1) with germylbromide **9** followed by LiHMDS-mediated amidation¹⁹ with *N*-aminomorpholine. The ethoxyethyl

group appended to the linker was present to mimic a PEG grafted polymer support. A range of conditions was evaluated for *ipso*-iododestannylation of this model system to give AM281 (**1c**, Table 1).

Table 1 Synthesis of and screening *ipso*-iododestannylation conditions on a solution phase germyl-AM281 precursor

entry	conditions ^a	yield (%)
1	NaI, chloramine T, AcOH, rt, 16 h	0 ^c
2	I ₂ , CCl ₄ , rt, 16 h	0 ^c
3	NaI, FeCl ₃ , rt, 16 h	0 ^c
4	ICl, rt, 16 h	0 ^c
5	NH ₄ I, Oxone [®] , rt, 16 h	0 ^c
6	NaI, <i>m</i> -CPBA, rt, 16 h ^b	0 ^d
7	NaI, AcO ₂ H, AcOH, rt, 16 h ^b	0 ^d
8	NIS, TFA (cat.), AcOH, rt, 16 h	0 ^d
9	I ₂ , AcOH, rt, 16 h	15 ^e
10	I ₂ , TFA, AcOH, rt, 16 h ^f	63 ^e
11	NaI, NCS, TFA, AcOH, rt, 30 min ^f	85

^a All reactions were carried out with 2 equiv of reagents. ^b slow addition of peracids ^c No reaction occurred; recovery of S.M. ^d Decomposition of S.M. ^e by-product **12** also isolated (see text). ^f TFA-AcOH (1:3 v/v).

Subjection of arylgermane **11** to the conditions reported by Gatley^{13d, 13f} for *ipso*-iododestannylation to give AM281 using NaI/chloramine-T/AcOH^{4a} or I₂/CCl₄^{4a, b} effected no reaction; starting material was recovered quantitatively (entries 1 and 2). Use of NaI/FeCl₃,²⁰ NH₄I/Oxone[®]²¹ and ICl^{4b} was similarly ineffective (entries 3-5). By contrast, use of NaI/*m*-CPBA,²² NaI/AcO₂H,²³ and NIS/TFA²⁴ resulted only in decomposition of the starting material, even with slow

²⁰ Mohanakrishnan, A.; Prakash, C.; Ramesh, N. *Tetrahedron* **2006**, 62, 3242.

²¹ Mohan, K. V. V. K.; Narrender, N.; Kulkarni, S. J. *Tetrahedron Lett.* **2004**, 45, 8015.

²² Sha, C.-K.; Young, J.-J.; Jean, T.-S. M. *J. Org. Chem.* **1987**, 52, 3919.

²³ Katsifis, A.; Mattner, F.; Zhang, Z.; Dikic, B.; Papazian, V. J. *Labelled Cpd. Radiopharm.* **2000**, 43, 385.

²⁴ Castanet, A.-S.; Colobert, F.; Broutin, P. E. *Tetrahedron Lett.* **2002**, 43, 504.

¹⁵ (a) Tobishi, S.; Sasada, T.; Nojiri, Y.; Yamamoto, F.; Mukai, T.; Ishiwata, K.; Maeda, M. *Chem. Pharm. Bull.* **2007**, 55, 1213, (b) Donohue, S. R.; Halldin, C.; Pike, V. W. *Bioorg. Med. Chem.* **2006**, 14, 3712, (c) Lan, R.; Liu, Q.; Fan, P.; Lin, S.; Fernando, S. R.; McCallion, D.; Pertwee, R.; Makriyannis, A. *J. Med. Chem.* **1999**, 42, 769, (d) Thomas, B. F.; Francisco, M. E. Y.; Seltzman, H. H.; Thomas, J. B.; Fix, E. E.; Schulz, A.-K.; Gilliam, A. F.; Pertwee, R. G.; Stevenson, L. A. *Bioorg. Med. Chem.* **2005**, 13, 5463.

¹⁶ (a) Amos, R. J. I.; Corulay, B. S.; Schiesser, C. H.; Smith, J. A.; Yates, B. F. *Chem. Commun.* **2008**, 1695, (b) Stieber, F.; Grether, U.; Waldmann, H. *Angew. Chem. Int. Ed.* **1999**, 38, 1073.

¹⁷ Turner, D. J.; Anemian, R.; Mackie, P. R.; Cupertino, D. C.; Yeates, S. G.; Turner, M. L.; Spivey, A. C. *Org. Biomol. Chem.* **2007**, 5, 1752.

¹⁸ Eaborn, C.; Pande, K. C. *J. Chem. Soc.* **1960**, 1566.

¹⁹ cf. Gustafsson, T.; Pontén, F.; Seeberger, P. H. *Chem. Comm.* **2008**, 1100.

addition of the oxidants (entries 6-8). However, the use of I_2 /AcOH, as described by Moerlein for *ipso*-iododegermylation of aryltrimethylgermanes,^{4b} resulted in a sluggish reaction (16 h) which yielded AM281 (**1a**) in 15% yield along with protonated by-product **12** in 10% yield (entry 9). Using TFA in place of AcOH gave an improved outcome: **1a** (63%), **12** (10%), but the reaction remained slow (16 h, entry 10). Further optimization of these conditions led to the use of NaI/NCS in a TFA/AcOH solvent system which gave AM281 (**1a**) in 85% yield with no by-product **12** observed, in just 30 min (entry 11). This protocol was considered sufficiently efficient to merit its deployment for our envisaged SPS for which we opted to employ the PEG-grafted, cross-linked polystyrene HypoGel[®]-200 as the polymer support on account of its favourable swelling characteristics in hydrophilic solvents.

HypoGel[®]-bound 1,5-diarylpyrazole *tert*-butyl ester **15** was prepared by an analogous protocol to that used for the solution model **10** but employing HypoGel[®]-bound germanium bromide **14** (0.75 mmol g⁻¹) for the transmetalation. Parallel amidation of functionalised polymer **15** with 3 × hydrazines and 2 × amines gave HypoGel[®]-bound 1,5-arylpyrazolyl hydrazides/amides **16a-e** (0.35-0.42 mmol g⁻¹, Table 2).

Table 2 Preparation of an array of HypoGel[®]-bound germyl arylpyrazolyl amides/hydrazides and their *ipso*-iododegermylation^a

entry	substrate	time	product	yield (%)
1	16a	1 h	AM281 (1c)	63
2	16b	1 h	AM251 (1b)	60
3	16c	1 h	17	60
4	16d	1 h	18	55
5	16e	6 h	19	42

6 **16b** 5 min **1b** 8
^a reaction conditions: **16** (1.0 equiv), NaI (5.0 equiv), NCS (4.0 equiv), TFA-AcOH (1 ml, 1:3 v/v), rt

Cleavage of the Ge linker with concomitant *ipso*-iodination was accomplished by mixing functionalized polymers **16a-e** with NaI in individual reaction cartridges, swelling the polymers in CH_2Cl_2 and then adding a solution of NCS in TFA/AcOH at rt. Iodinated rimonabant analogues **1c**, **1b** and **17-19** were then isolated in 42-63% yields (based on polymer loading levels) following removal of the polymer by filtration and then flash chromatography (entries 1-5). The reactions in entries 1-4 were complete within 1 h whereas that of entry 5 (the 4-fluoroanilino amide) required 6 h to reach completion. Exposure of polymer **16b** to the iodination conditions for just 5 min furnished AM251 (**1b**) in 8% yield after flash chromatography (entry 6). Analysis by HPLC with UV detection at 254 nm of the *crude* reaction mixture in the case of entry 2 revealed a purity of 80% due to contamination with succinimide (from the NCS). This impurity could be removed by briefly shaking the crude product mixture with a hydrazine functionalized Si-propyl[®] scavenger resin then filtration; this gave AM251 (**1b**) in a purity of 96% without recourse to chromatography (see Supporting Information). These findings augur well for adaption of our approach to radioiodination in a time-critical imaging context with the prospect of in-line purification through a scavenger cartridge for clinical application.²⁵

In conclusion, a novel protocol for the rapid parallel SPS of iodinated 1,5-diarylpyrazole CB₁ receptor ligands has been developed. The method features the use of a Ge linker which is both robust enough to allow on-resin diversification by LiHMDS-mediated amidation and reactive enough to allow rapid and clean *ipso*-iododegermylative cleavage. In particular, the method obviates the use of toxic and labile arylstannane congeners in solution as is presently widely practiced in this type of chemistry. We believe the method holds promise for application in the development of radioiodinated CB₁ receptor ligands as candidate PET/SPECT imaging agents for studying a range of CNS disorders and could be adapted to expedite development of other PET imaging ligands.

Acknowledgment. We thank the BBSRC and sanofi-aventis for provision of a studentship (TCJ).

Supporting Information Available. Experimental procedures and characterization for compounds/resins **1-19**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

²⁵ The preparation of no-carrier-added radioiodinated ligands requires very rapid kinetics even when employing a deficiency of the iodine donor at low concentration and adsorption of the labelled product onto glass/plastic reaction vessels can be problematic. We have not yet adapted the cleavage conditions described here for this scenario but envisage that development of an appropriate microfluidic flow-cell should allow these potential problems to be circumvented.