ORGANIC LETTERS

2000
Vol. 2, No. 8
1121−1124

Synthesis of Analogues of (–)-Cytisine for in Vivo Studies of Nicotinic Receptors Using Positron Emission Tomography

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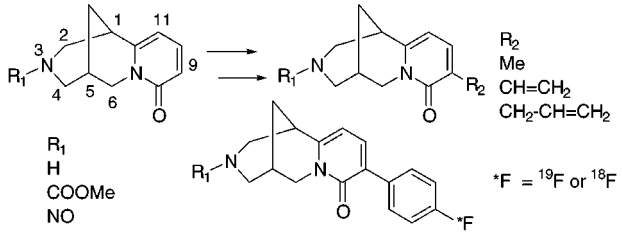
Received February 18, 2000

ABSTRACT



9-Substituted analogues of (–)-cytisine were synthesized in high yields via palladium-mediated couplings of either 9-(–)-bromocytisine and organostannanes or 9-(–)-trimethylstannylcytisine and fluorobromobenzene. The protection of the amine with a nitroso group and the use of $PdCl_2(PPh_3)_2$ to carry out the Stille reaction allowed the rapid synthesis of 9-(4′-[^{18}F]fluorophenyl)cytisine (^{18}F: $t_{1/2}$ = 109.7 min), a new promising radioligand (radiochemical yield: 10% from [^{18}F]KF, 150 min, four steps) for positron emission tomography studies of $\alpha_4\beta_2$ nicotinic receptors.

Nicotinic acetylcholine receptors (nAChRs) are involved in the various physiological effects observed with nicotine. Accumulating evidence suggests that compounds that activate neuronal nAChRs may be of therapeutic value.[1] Moreover, the nAChR densities are altered in various pathologies such as Alzheimer's and Parkinson's diseases.[2] As a result, the in vivo quantitation of receptors using positron emission tomography (PET)[3] has attracted tremendous interest as a tool to investigate the role of the nicotinic system in neurodegenerative diseases.[4] Recently, several radioligands have been prepared to visualize nAChRs in vivo by PET[5] or single photon emission tomography (SPET).[6] However, due to their toxicity (i.e., [^{18}F]fluoroepibatidine) or their high nonspecific binding (i.e., *N*-[^{11}C]methylcytisine), none of these ligands are ideally suited for PET imaging studies of the human brain. It is therefore crucial to develop a

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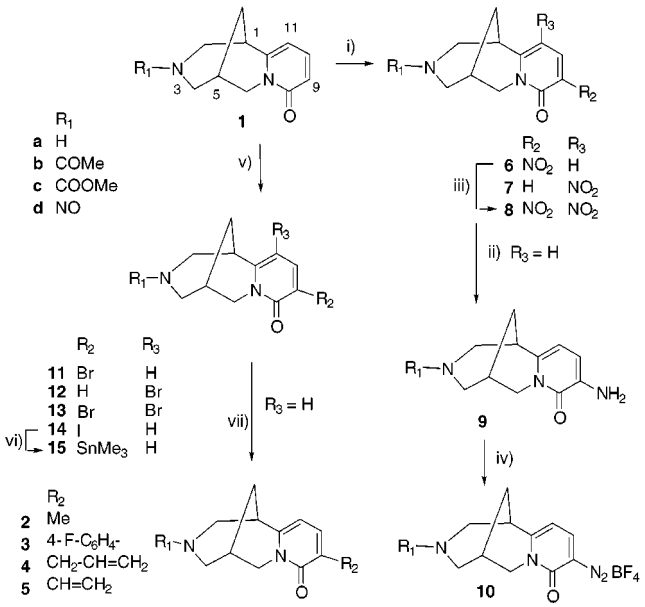
10.1021/ol005685m CCC: $19.00
Published on Web 03/23/2000

radiotracer to allow for mapping of these receptor sites in vivo. With this aim, we have envisaged structural modifications of (−)-cytisine **1a** (Scheme 1). Surprisingly, the

Scheme 1



(i) (a) H_2SO_4, HNO_3, rt, 5 h, **6a**: 77%; **7a**: 11%. (b) Ac_2O, DMAP, pyridine, rt, 12 h; **6b**: 100%. (ii) H_2, Pd/C, MeOH, rt, 4 h, **9b**: 100%. (iii) NH_4NO_3, TFAA, $CHCl_3$, rt, 16 h, **8b**: 70%. (iv) $NaNO_2$, HBF_4; **10b**: 71%. (v) NBS, DMF, 30 min, rt; **11c**: 57%, **12c**: 17%, **13c**: 2%; NBS, CH_2Cl_2, 4 h, 0 °C; **11d**: 50%; CF_3COOAg, I_2, CH_2Cl_2, 0 °C then rt, 12 h; **14c**: 55%; **14d**: 50%. (vi) $(Me_3Sn)_2$, $Pd(PPh_3)_4$, dioxane; **15d**: 70%. (vii) Pd^0, HMPA or dioxane (cf Table 1).

chemistry of this alkaloid, extracted from many Leguminosae,[7] is not well documented. It has been limited until recently[8,9] to reactions on the secondary amine and to a few electrophilic substitutions on the pyridone ring.[10]

Owing to its nanomolar affinity[11] and its high selectivity toward the $\alpha_4\beta_2$ receptor subtypes,[12] (−)-cytisine is often used as a reference ligand in the studies of the nicotinic neurotransmission. Its long half-life in vivo compared to that of nicotine,[13] and its ability to cross the blood-brain barrier,[14] make cytisine a good candidate for PET studies.[15]

Carbon-11 ($t_{1/2}$: 20.4 min) and fluorine-18 ($t_{1/2}$: 109.7 min) are the most common positron emitters used to develop PET radiotracers. The difficulty in designing a rapid synthesis of [^{11}C]cytisine and the need to keep unprotected the secondary amine function of cytisine led us to develop first the synthesis of 9-methylcytisine **2a**. Since the time for cytisine to reach a maximum concentration in mouse brain is relatively long (30 min),[15] the syntheses of 9-(4′-fluorophenyl)cytisine **3a** and allyl and vinyl cytisine **4a** and **5a**, respectively (Scheme 1), precursors of fluoroethyl or fluoropropyl chains, were also envisaged. Possible hypoglycemic and antiinflammatory activities of cytisine[16] and the development of cytisine analogues for the treatment of neurodegenerative deseases[8] prompted us to report our preliminary results on the synthesis of pyridone-substituted cytisines and on the radiosynthesis of 9-(4′-[^{18}F]-fluorophenyl)cytisine [^{18}F]**3a**.

The most common method for introducing a fluorine-18 atom onto an aromatic ring is nucleophilic substitution of a nitro (or trimethylammonium) group for a fluoride.[17] 9-Nitrocytisine **6b** (R_1 = COMe, R_3 = H) and 9,11-dinitrocytisine **8b** (R_1 = COMe, R_3 = NO_2) were prepared in 77% and 54% yields from (−)-cytisine easily extracted from *Laburnum anagyroides* seeds.[18] All attempts of radiofluorinations ([^{18}F]KF/K_{222}, DMSO, 15 min, 150 °C) of these nitro compounds **6b** and **8b** failed.

The Balz−Schiemann reaction is another route for synthesizing fluoroaromatic or heteroaromatic compounds,[19] and the pyrolysis of [^{18}F]tolyldiazonium fluorotrichloroborate was shown to be a possible route to [^{18}F]fluorotoluene,[20] at a no carrier added state. Tetrafluoroborate **10b** was prepared from the corresponding amine **9b**. Its heating neat[21] or in the presence of KF[22] at 200 °C, under reflux of xylene,[23] dichloromethane,[24] DMSO, and HBF_4, or in the presence of copper,[25] gave only tars.

Less than 10% of cytisine **1a** was formed from reduction of the diazo group. The presence of a carbonyl group ortho to the diazonium group could explain this thermal behavior.[26] These results led us to develop the preparation of fluoroalkyl or aryl derivatives of cytisine.

Introduction of an alkyl, alkenyl, aryl, or heteroaryl group to the carbonyl group of 2-pyridones is not well documented.

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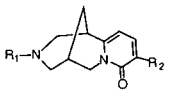
Table 1. Cross-Coupling Reactions of Organostannanes with 9-Halocytisine

Entry	 R_1	R_2	Starting Material	Reagent	Catalyst Ligand or additive	Solvent	Temp (°C)	Time (h)	Product	Yields (%)
1	NO	I	**14d**	Me_4Sn	$ClPdBn(PPh_3)_2$	HMPA	60	12	**2d**	25
2	NO	I	**14d**	Me_4Sn	$PdCl_2(MeCN)_2$	"	60	24	**2d**	23
3	NO	Br	**11d**	Me_4Sn	$ClPdBn(PPh_3)_2$	"	60	24	**2d**	60
4	NO	Br	**11d**	Me_4Sn	$ClPdBn(PPh_3)_2$	"	80	12	**2d**	60
5	NO	Br	**11d**	Me_4Sn	$ClPdBn(PPh_3)_2$	"	120	0.25	**2d**	81
6	NO	Br	**11d**	Bu_3Sn(allyl)	$ClPdBn(PPh_3)_2$	"	120	0.5	**4d**	55
7	NO	Br	**11d**	Bu_3Sn(vinyl)	$PdCl_2(PPh_3)_2$	dioxane	120	1	**5d**	70
8	NO	Br	**11d**	Bu_3Sn(vinyl)	$PdCl_2(MeCN)_2$	HMPA	60	48	**5d**	(100)[a]
9	NO	I	**14d**	$Sn(vinyl)_4$	$PdCl_2(MeCN)_2$	"	60	12	**5d**	(100)[a]
10	CO_2Me	Me_3Sn	**15c**	4-F-C_6H_4-Br	$Pd_2(dba)_3$, $AsPPh_3$	dioxane	101	60	**3c**	37
11	CO_2Me	Me_3Sn	**15c**	4-F-C_6H_4-Br	$PdCl_2(PPh_3)_2$, LiCl	"	101	12	**3c**	72
12	NO	Me_3Sn	**15d**	4-F-C_6H_4-Br	$PdCl_2(PPh_3)_2$, LiCl	"	101	12	**3d**	68

[a] In parentheses: not isolated. HPLC showed the complete conversion of the starting material to **5d**.

The Friedel−Crafts reaction[27] and UV irradiation in the presence of a heterocyclic compound[28] have been reported along with a few Heck reactions[29] and Stille[30] and Suzuki[31] couplings. With these palladium-catalyzed reactions of halides with organometallic compounds being some of the most straightforward methods currently available for carbon−carbon bond formation,[32] the cross-coupling reactions of 9-halogeno- or 9-trimethylstannylcytisine was envisaged for the preparation of the target compounds. The *N*-protected 9-bromocytisines **11c** and 11-bromocytisines **12c** were obtained in 57 and 17% yields, respectively, by reaction of cytisine **1c** with *N*-bromosuccinimide (NBS) at 0 °C. The ratio of the 9- and 11-regioisomers was shown to be strongly dependent on the solvent [relative ratio **11c**/**12c**/**13c**: 73/23/2 (DMF), 72/19/9 (MeCN), 65/31/4 (CH_2Cl_2), 75/18/5 (H_2O, H^+), 85/15/0 (THF)]. Similar results were obtained when *N*-nitrosocytisine **1d** was treated with NBS. *N*-Nitroso 9-iodocytisine **14d** was prepared in 50% yield by treatment of the *N*-protected cytisine **1d** with iodine in the presence of silver trifluoroacetate.[33] Partial biological evaluation[34] of the synthesized compounds showed that the 9-substituted derivatives have a higher affinity toward the $\alpha_4\beta_2$ nAChRs than their 11-regioisomers.

The reaction of 9-iodo- or 9-bromocytisines **14d** or **11d** with tetramethyltin, tri-*n*-butylallyltin, tri-*n*-butylvinyltin, or tetravinyltin were carried out at different temperatures (60−120 °C), times (0.25−48 h), and solvents (HMPA, DMF, dioxane) using $ClPdBn(PPh_3)_2$, $PdCl_2(MeCN)_2$, or $PdCl_2(PPh_3)_2$ as the catalyst (Table 1). *N*-Nitroso-9-methylcytisine **2d** was obtained in more than 80% yield when the cross-coupling reaction of tetramethylstannane with the bromo compound **11d** was carried out at 120 °C using a short reaction time (15 min) (entry 5). Under the same conditions (entry 6), the coupling of tri-*n*-butylallylstannane with **11d** was less efficient (55%), but no conjugaison of the double bond was observed. The use of $PdCl_2(PPh_3)_2$ led to 9-vinylcytisine **5d** in 70% yield (entry 7). Finally, reaction of iodocytisine **14d** with hexamethylditin in dioxane in the presence of $Pd(PPh_3)_4$[35] yielded stannylcytisine **15d**. The cross-coupling reaction of **15d** with 4-fluorobromobenzene afforded 9-(4′-fluorophenyl)cytisine in yields of up to 65−70%. The use of $PdCl_2(PPh_3)$ in the presence of an excess of LiCl[36] (Table 1, entries 11 and 12) was shown to be superior to $Pd_2(dba)_3$/$AsPPh_3$.[37]

The incorporation of ^{18}F into organic compounds presents many challenges including the need to synthesize and to purify the compounds within a 2−3 h time frame and the need to work on a microscale. Moreover, PET studies using high specific activity tracers require the use of [^{18}F]fluoride as the fluorine-18 source. Recently, we and others[38] showed

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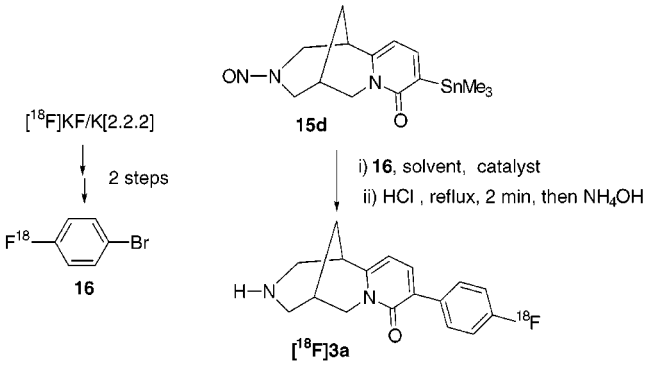
Scheme 2



the possibility of carrying out rapid and efficient Stille couplings with 4-[^{18}F]fluorobromobenzene [^{18}F]**16**. This led us to study the synthesis of 9-(4′-fluorophenyl)cytisine [^{18}F]-**3a** using the strategy depicted in Scheme 2. 4-[^{18}F]Fluorobromobenzene was prepared in two steps from [^{18}F]KF/Kryptofix222.[38] Its reaction with **15d** was studied under various conditions (Table 2).

Table 2. Cross-Coupling Reactions of 4-[^{18}F]Fluorobromobenzene and 9-Trimethylstannylcytisine **15d**

entry	catalyst	solvent[a]	time (min)	RCY (%)[b]
1	$Pd_2(dba)_3$ $AsPh_3$	DMF, dioxane	10	27
2	$Pd(PPh_3)_4$	DMF, dioxane	10	68
3	$PdCl_2(PPh_3)_2$	dioxane	15	56−74[c]

[a] All the reactions were carried out at 110 °C. [b] Radiochemical yield decay corrected from 4-[^{18}F]fluorobromobenzene. [c] Three runs.

The results are in good agreement with those obtained using stable isotope chemistry with much shorter reaction times (10−15 min vs 12−60 h).[39] Rapid denitrosation of [^{18}F]**3d** afforded 9-(4′-[^{18}F]fluorophenyl)cytisine [^{18}F]**3a** in 6−10% radiochemical yield, corrected for decay (150 min total time synthesis) from [^{18}F]KF. Work is in progress to measure the specific radioactivity of this new radiotracer.[40]

In summary, we have reported an easy transformation of cytisine to a range of 9-substituted derivatives. The key step was the Stille cross-coupling reaction of halogeno- or tin-substituted cytisine derivatives. The reaction can be performed under very constraining conditions compatible with the use of short-lived isotopes: short reaction times, submicromolar amounts of the heteroaryl bromide, large excess of reagents, and very rapid and efficient removal of a nitroso protecting group. Using those conditions we have prepared 9-(4′-[^{18}F]fluorophenyl)cytisine, which will allow the in vivo study of $\alpha_4\beta_2$ nicotinic receptors via PET imaging. Compounds **11** and **15** described here will be used as precursors for the synthesis of 9-([^{11}C]methyl)cytisine. Vinyl and allyl cytisines **4** and **5** will be functionalized in order to access 9-([^{18}F]fluoroethyl)- and 9-([^{18}F]fluoropropyl)cytisines.

Acknowledgment. This work was supported by the Réseau Interrégional de Chimie Organique Fine Action 7 (Contrat de Plan Etat Bassin Parisien, Régions Haute Normandie, Basse Normandie). The authors thank Jean Christophe Plaquevent and Dominique Cahard (University of Rouen) for helpful discussions and Samir Jegham and Olivier Curet for biological evaluations (Synthelabo, France).

Supporting Information Available: Full experimental details for the syntheses reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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(39) *N*-Nitroso 9-(4-[^{18}F]fluorophenyl)cytisine and 9-(4-[^{18}F]fluorophenyl)cytisine were characterized by comparison of their R_f or retention times with those of authentic samples in radio-TLC and/or HPLC.

(40) The radioactive experiments were carried out with 185 MBq of [^{18}F]-KF. Under these conditions, no attempt was made to measure the specific radioactivity.