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Synthesis of the first sulfur-35-labeled hERG radioligand

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Abstract—The synthesis of the first high specific activity S-35-labeled hERG radioligand, [^{35}S]MK-0499, for use in HTS assays of drug candidates for hERG interaction is described. The radioligand is prepared by [^{35}S]sulfonylation of a high diastereomeric excess (de) aniline precursor prepared from unlabeled MK-0499.

Acquired long QT syndrome is a potentially lethal cardiac condition that can result from heart disease, natural cardiac defects, or from inhibition of I_{Kr}, the rapid delayed rectifier K^+ current in human ventricular myocardium, by an increasing number of drugs. The human *ether-à-go-go*-related gene K^+ channel protein (hERG) is the pore-forming subunit that underlies I_{Kr}, which is crucial to the repolarization of cardiac tissue.[1–4] Compounds shown to elicit QT prolongation have widely varying chemical structures and mechanisms of action.[5] Late detection of this side effect may have hazardous consequences for patients, and could increase drug development costs greatly.[6] As a result, screening of new drug candidates for I_{Kr} activity using hERG radioligands is a very important approach to eliminating compounds with this potential early in the drug development process. Recently, high throughput assays used to screen for compounds of this type have been developed that employ the binding of tritium-labeled methanesulfonanilide I_{Kr} blockers MK-0499 **1** and dofetilide **2** as hERG radioligands,[7] together with membranes from cells that recombinantly express hERG protein (Fig. 1).[8]

We report here the synthesis of high specific activity [^{35}S]MK-0499, the first S-35-labeled hERG radioligand, for use in high throughput screening (HTS) assays of drug candidates for interaction with hERG.[9,10] Sulfur-35-labeled radioligands typically have a significantly higher specific activity (up to ~1400 Ci/mmol) than that of tritiated radioligands (up to ~28 Ci/mmol per tritium), allowing smaller quantities of radioligand and lower hERG concentrations to be used, and more sensitive assays to be developed. MK-0499 itself is a potent and selective I_{Kr} blocker which inhibits the native cardiac I_{Kr} current and its heterologously expressed α-subunit, hERG, with IC_{50} values of 44 and 21 nM, respectively.[11–13]

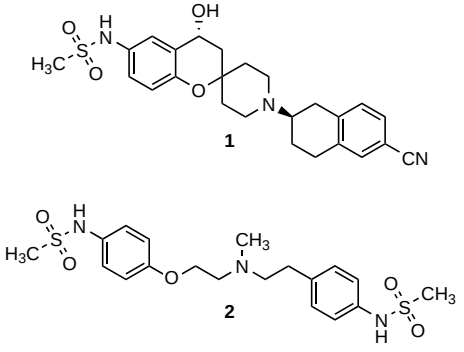

Figure 1. Methanesulfonanilide I_{Kr} blockers.

Our initial attempts at synthesis of **1** were based on the availability of a small quantity of ketoaniline **3**, a

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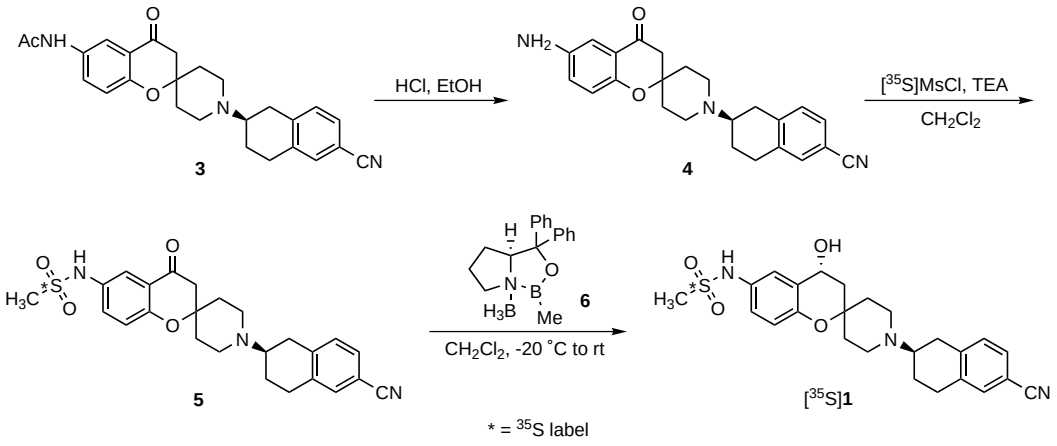


Scheme 1. Synthesis of [^{35}S]MK-0499 via chiral reduction.

process intermediate (Scheme 1).[14] Deprotection of **3**, followed by sulfonylation with [^{35}S]methanesulfonyl chloride using conditions similar to those described by Dean et al.,[15] gave [^{35}S]ketone **5**. Stereoselective reduction of **5** using chiral oxazaborolidine–borane complex **6** yielded [^{35}S]MK-0499.[16] HPLC analysis to determine the diastereoselectivity of the high specific activity reduction did not give baseline resolution of the diastereomers directly. Derivatization as the Mosher ester allowed separation of the diastereomers on a Cosmosil 5PYE HPLC column, but was problematic at high specific activity. Using this method, the diastereomeric excess (de) was found to vary unacceptably from batch to batch (80–95% de). The variable stereoselectivity, difficult de determination, and limited supply of **3** made this route unsuitable for routine synthesis of the radioligand to support the HTS assay.

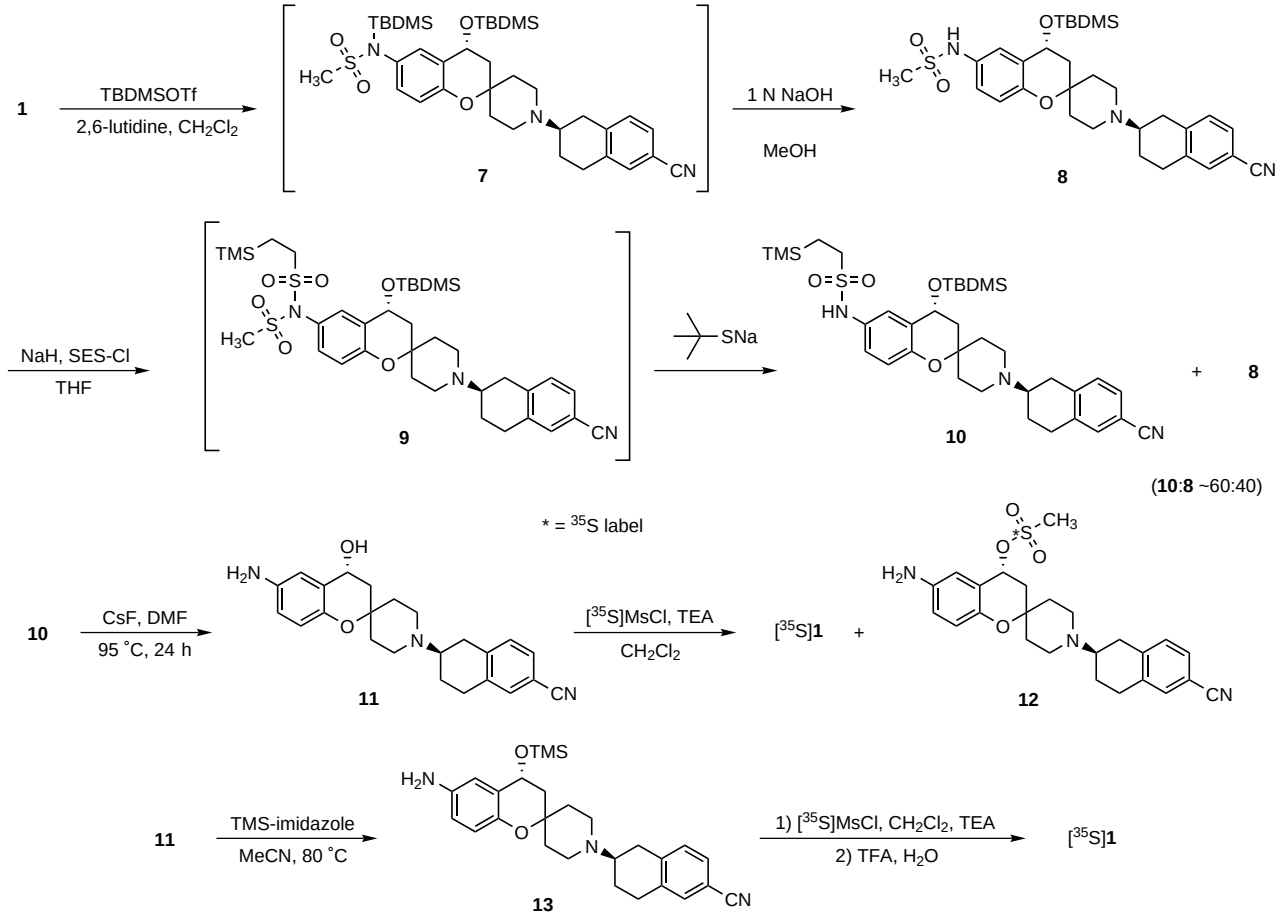
We then investigated alternative approaches that would eliminate the chiral reduction step through use of a diastereomerically pure hydroxyaniline labeling precursor. Since large supplies of unlabeled MK-0499 were available, desulfonylation to yield a precursor of this type was an attractive option. Subsequent re-sulfonylation with [^{35}S]mesyl chloride would give, overall, conversion of unlabeled MK-0499 to [^{35}S]MK-0499. Conditions for cleavage of aryl sulfonamides are typically harsh,[17] prompting us to consider activating it as a sulfonimide which is more easily hydrolyzed.[18] Our strategy was to prepare a mixed sulfonimide of **1** by sulfonylation with trimethylsilylethanesulfonyl chloride (SES-Cl),[19] with the intention of hydrolyzing the methanesulfonamide selectively under basic conditions, followed by trimethylsilylethanesulfonamide cleavage with fluoride ion.

This required initial protection of the alcohol, which was achieved by silylation with 2.5 equivalents of TBDMS triflate to give bis-protected intermediate **7** (Scheme 2). Use of only 1.1 equivalents of silylating reagent gave a mixture of mono- and bis-silylated products, as well as unreacted starting material. Crude **7** was selectively *N*-deprotected with 1 N NaOH/MeOH to give TBDMS ether **8** in 88% yield from **1** after silica gel purification. The methanesulfonamide was then activated by sulfonylation with SES-Cl, to give sulfonimide **9**. We then investigated the selective cleavage of the methanesulfonamide of **9** over the trimethylsilylethanesulfonamide by quenching the reaction mixture with various nucleophiles. Use of a thiol nucleophile appeared to be important in obtaining some selectivity for methanesulfonamide cleavage. Quenching of the reaction mixture with 1 N NaOH resulted in selective cleavage of the trimethylsilylethanesulfonamide to give only **8**, while use of sodium thiomethoxide or 2-propanethiolate gave a 40:60 ratio of products **10**:**8**. Use of 5 equivalents of the bulkier sodium 2-methyl-2-propanethiolate gave the best product yield (~60:40 ratio of **10**:**8**). Silica gel chromatography yielded pure **10** in 50% yield and allowed recovery of **8** for recycling. Any contamination of **10** with **8** would, after deprotection, generate unlabeled **1**, which could lower the specific activity of the final [^{35}S]**1** to unacceptable levels. Careful chromatographic purification of **10** to remove **8** to the level of <20 ppm (determined by LC–MS) was thus essential.[20]

Purified **10** was then subjected to simultaneous desilylation/sulfonamide cleavage using 3 equivalents of CsF to provide hydroxylaniline **11**. Mesylation of **11** with [^{35}S]mesyl chloride gave [^{35}S]**1** in 16% radiochemical yield, a major radioactive by-product (12%) suspected to be the *O*-mesylated product **12**, as well as [^{35}S]methanesulfonic acid (~70%).

To prevent this, the alcohol was protected by silylation with TMS-imidazole to give trimethylsilylether **13**. [^{35}S]Mesylation of **13** using standard conditions gave the corresponding *O*-TMS-protected [^{35}S]sulfonamide in a more satisfactory 48% radiochemical yield ([^{35}S]MsOH accounted for the remaining radioactivity).[21] After deprotection with TFA and HPLC purification, [^{35}S]MK-0499 was isolated in 35% overall radiochemical yield. The specific activity was determined to be 1198 Ci/mmol by LC–MS, in agreement with the known levels of unlabeled **1** carried into the labeling reaction.[22]

Finally, it remained to be determined whether the 8-step conversion of unlabeled **1** to [^{35}S]**1** had resulted in



Scheme 2. Synthesis of [^{35}S]MK-0499 from unlabeled MK-0499.

epimerization of the benzopyranyl chiral center. Due to the difficulty of the established Mosher ester derivatization of the radioligand and reversed-phase HPLC analysis of the diastereomers, other methods for separating the diastereomers of **1** directly were investigated. Screening of chiral HPLC columns led to the development of a normal phase method using a Chiralpak AS column that provided baseline resolution of the diastereomers.[23] Using this assay, the de of [^{35}S]**1** prepared by [^{35}S]mesylation of **13** and subsequent deprotection was found to be 98%, showing there was no significant loss of optical purity during the conversion of **1** to [^{35}S]**1**.

In summary, a practical synthesis of high specific activity [^{35}S]MK-0499 from unlabeled MK-0499 has been developed. In the process, a procedure for the mild cleavage of the methanesulfonamide of **1** has been demonstrated that ultimately produced key hydroxyaniline sulfonylation precursor **13** with high de and low contamination with unlabeled methanesulfonamide-containing impurities. [^{35}S]Sulfonylation of **13**, followed by deprotection, gave [^{35}S]**1** in good radiochemical yield, with high specific activity and de. The relatively large supply of **13** synthesized has allowed for the routine preparation of ~20 mCi batches of [^{35}S]MK-0499 to support the high throughput screening of drug candidates for hERG interaction.

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20. Material purified by flash chromatography using 1/1 ethyl acetate/hexane. Combining fractions that contained 20 ppm or less of **8** by LC–MS analysis yielded 1.43 g (50%) of **10**. Further elution with 99/1 ethyl acetate/methanol allowed recovery of ~1.1 g of **8**.
21. Synthesis of [^{35}S]MK-0499 by sulfonylation of **13**: [^{35}S]Methanesulfonate (SA usually >1200 Ci/mmol) was purchased from PerkinElmer and converted to [^{35}S]mesyl chloride using the procedure of Dean et al (see Ref 15). A solution of [^{35}S]mesyl chloride (40 mCi) in CH_2Cl_2 was concn to ~250 μl by atmospheric distillation at ~60 °C in a short-path distillation apparatus. After cooling to room temperature, the residue was transferred to a solution of **13** (12 mg) in anhyd CH_2Cl_2 (30 μl) via blunt-end syringe. Anhyd Et_3N (10 μl) was then added, and the reaction mixture was stirred at room temperature for 2 h. The reaction was assayed by RP HPLC after quenching of an aliquot into 2:1 MeCN/H_2O. (Zorbax RX-C18 4.5 × 250 mm analytical column, 30 °C, 210/254 nm, 1 ml/min, B = MeCN, A = 0.1% aq $HClO_4$, 20B:80A linear gradient to 100B over 20 min, hold for 10 min, t_R [^{35}S]MsOH = 3.1 min, t_R **13** = 8.6 min, and t_R sulfonamide = 11.4 min, 48% sulfonamide). The reaction mixture was diluted with H_2O (200 μl), and TFA (10 μl) was added to deprotect the *O*-TMS groups. After stirring for 1 h at room temperature, the reaction was diluted with CH_2Cl_2 (3 ml) and H_2O (1 ml), and stirred vigorously. The CH_2Cl_2 layer was separated and washed with saturated $NaHCO_3$ (2× 2 ml), and the aqueous layer was extracted with CH_2Cl_2 (3× 1 ml). The combined organic layers were dried over Na_2SO_4, filtered, and concd under vacuum. The residue was dissolved in MeCN/50% aq TFA (2:1) and purified by sequential semi-preparative HPLC (Method 1: Zorbax RX-C8 9.4 × 250 mm column, 215 nm, 4 ml/min, B = MeCN, A = 0.1% aq $HClO_4$, 25B:75A isocratic, t_R amine = 7 min, t_R [^{35}S]MK-0499 = 25 min; Method 2: Phenomenex Prodigy Phenyl-3 9.4 × 250 mm column, 215 nm, 4 ml/min, B = MeCN, A = 0.1% aq $HClO_4$, 28B:72A isocratic, t_R [^{35}S]MK-0499 = 32 min). The product was isolated by SPE and formulated in MeOH, yielding 14 mCi of [^{35}S]MK-0499 with >99% radiochemical purity by HPLC (Phenomenex Prodigy Phenyl-3 4.5 × 250 mm analytical column, 30 °C, 210/254 nm,1 ml/min, B = MeCN, A = 0.1% aq $HClO_4$, 33B:67A isocratic for 20 min, hold for 10 min, t_R [^{35}S]MK-0499 = 15.6 min). Identity was confirmed by co-elution with authentic standard. LC–MS: *m/z* 471 $[M+H]^+$ for S-35 labeled, 468 $[M+H]^+$ for unlabeled, specific activity = 1198 Ci/mmol.
22. Compounds **8**, **10**, **11**, and **13** were characterized by ^{1}H NMR and/or LC–MS (ESI).
23. The HPLC method used to separate the diastereomers was: Chiralpak AS 5 μm, 4.6 × 250 mm analytical column, 235 nm, 1 ml/min, A = 0.1% *i*Pr_2NH in hexanes, B = 0.1% *i*Pr_2NH in 1:1 EtOH/MeOH, 80A:20B linear gradient to 50A:50B over 30 min, t_R correct diastereomer = 11.5 min, t_R incorrect diastereomer = 14.5 min.