Note

Journal of Labelled Compounds and Radiopharmaceuticals

Received 28 August 2013, Revised 25 September 2013, Accepted 11 October 2013 Published online 26 November 2013 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3138

Syntheses of [$^3H_2$]T0901317 and a labeled structural isomer, and characterization of the dispersed labeled compounds via ^{19}F NMR

Benjamin P. Fauber,[a]* Adam R. Johnson,[a] Stephen Bowerman,[c] Brenda Burton,[b] Adam Colebrook,[c] Annerley Flynn,[c] Gareth Harrold,[c] Steve Huhn,[a] Gary Jones,[c] Peter Lockey,[b] Maxine Norman,[b] Olivier René,[a] and Harvey Wong[a]

The synthesis and characterization of [$^3H_2$]T0901317 and a structural isomer are described. The structural assignments of the closely related labeled compounds were primarily accomplished via ^{19}F NMR analyses of the corresponding ethanolic compound dispersions.

Keywords: T0901317; tritium; ^{19}F NMR; LXR; PXR; RORγ; RORc

Introduction

The benzenesulfonamide compound T0901317 (Figure 1, Compound **1**) was initially disclosed by a team at Tularik (now Amgen) as a potent liver X receptor (LXR) agonist.[1] [^{3}H] T0901317 (specific activity = 27 Ci/mmol, Amersham) has since been used to probe the binding of small-molecule ligands to the nuclear receptors LXR,[2] pregnane X receptor,[3] and retinoic acid receptor-related orphan receptor gamma.[4] Although [^{3}H] T0901317 has been reported as an assay tool, to our knowledge, the synthesis and characterization of this radioligand have not been disclosed.

It is well recognized that increasing the specific activity of a radioligand can improve the signal-to-noise ratio when the ligand is used in a radiometric assay. With such a consideration in mind, we sought to synthesize [$^3H_2$]T0901317 as a radioligand for use in nuclear receptor radiometric binding assays.

Experimental

Reaction mixtures were analyzed for consumption of starting material on an Agilent 1100 series LCMS using a linear gradient of 0.1% trifluoroacetic acid in water and acetonitrile mobile phases and a Waters X-bridge C18 column (2.5 μm, 3 × 30 mm). ^{1}H, ^{3}H, and ^{19}F NMR spectra were recorded in DMSO-d_6 solutions using a Bruker AV-400 spectrometer or a Bruker AV-300 spectrometer with tetramethylsilane, or residual solvent, as an internal reference for the ^{1}H NMR spectra and trifluoroacetic acid as an internal reference for the ^{19}F NMR spectra (−76.6 ppm). The ^{3}H NMR spectrum was referenced to the corresponding ^{1}H NMR proton frequency of the *para*-hydrogen on the sulfonamide aromatic ring. High-resolution mass spectra were obtained on a Kratos Concept IIH using positive or negative electrospray ionization. Flash column chromatography purification was performed using a CombiFlashRf Teledyne ISCO instrument with SiliCycle SiliaSepHP (15–45 μm mesh size) or ISCO RediSepRf Gold (20–40 μm mesh size) silica gel cartridges. Unless otherwise specified, all reagents and solvents were used as is from commercial sources.

The scintillation proximity assay (SPA) format was used to detect binding of the synthesized radioligands **7** and **8** to the nuclear receptor LXRα. Incubations were carried out using 50 nM N-terminal GST-tagged LXRα ligand binding domain (Life Technologies, PV4657) and 25 nM radioligand. Yttrium silicate polylysine-coated SPA beads (PerkinElmer, RPNQ0010) were used at 0.5 mg/well in 96-well format and a reaction volume of 50 μL. The incubation buffer was as described,[5] except we used a pH of 7.4. Total binding (TB) was determined as the signal in the presence of 50 nM LXRα receptor and 25 nM radioligand. Nonspecific binding was determined in incubations of 50 nM LXRα, 25 nM radioligand, and 3 μM unlabeled T0901317 (Sigma-Aldrich, T2320). All SPA binding mixtures were incubated at room temperature, and the SPA signal (cpm) was measured after 10, 30, 60, 120, 180, and 240 min using a MicroBeta plate reader (PerkinElmer). Specific binding (SB) was calculated by subtracting the signal for nonspecific binding from the signal for TB, while the percent SB (%SB) was calculated using the equation: %SB = [(SB/TB) × 100].

2,4-dibromo-*N*-(4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl)benzenesulfonamide (4)

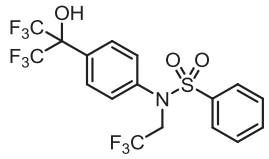
2-(4-aminophenyl)-1,1,1,3,3,3-hexafluoro-propan-2-ol (**2**) (1.5 g, 5.8 mmol), 2,6-lutidine (1.0 mL, 8.7 mmol), acetone (29 mL), and 2,4-dibromobenzenesulfonyl chloride (**3**) (2.1 g, 6.4 mmol) were combined and stirred at

[a]*Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA*

[b]*Argenta, Units 7-9 Spire Green Centre, Flex Meadow, Harlow, Essex CM19 5TR, UK*

[c]*Quotient Bioresearch, The Old Glassworks, Nettlefold Road, Cardiff, Wales, CF24 5JQ, UK*

**Correspondence to: Benjamin P. Fauber, Department of Discovery Chemistry, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA. E-mail: fauber.benjamin@gene.com*



1

Figure 1. Structure of T0901317.

23°C. The reaction was heated at reflux for 36 h. The reaction was cooled to 23°C and quenched with EtOAc (30 mL) and 10% (w/v) aqueous KH_2PO_4 (2 × 30 mL). The organic fraction was washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was triturated with heptane, and the resulting solid was subjected to column chromatography (SiO_2, 10–30% EtOAc in heptane) to yield the title compound (**4**) as a white solid (2.58 g, 80% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.08 (s, 1H), 8.57 (s, 1H), 8.13 (d, J = 4 Hz, 1H), 8.03 (d, J = 12 Hz, 1H), 7.81 (dd, J = 8, 4 Hz, 1H), 7.54 (d, J = 8 Hz, 2H), 7.20 (d, J = 8 Hz, 2H); ^{19}F NMR (282 MHz, DMSO-d_6): δ −75.5 (s, 6F). MS ESI (m/z): [M-H]$^-$ calcd for $C_{15}H_8Br_2F_6NO_3S$, 556.1; found, 556 and 558 (dibromo pattern).

2,4-dibromo-*N*-(4-(1,1,1,3,3,3-hexafluoro-2-(2,2,2-trifluoroethoxy)propan-2-yl)phenyl)benzenesulfonamide (5)

2,4-dibromo-*N*-(4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl) benzenesulfonamide (**4**) (1.3 g, 2.33 mmol), 1,1,1-trifluoro-2-iodoethane (2.2 g, 10.4 mmol), and K_2CO_3 (635 mg, 4.6 mmol) were combined in DMF (15 mL) and stirred at 100°C for 18h. The reaction mixture was cooled to 23°C and poured into water (100 mL). The mixture was extracted with EtOAc (3 × 100 mL). The organic fractions were combined and washed with brine (250 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, 15% EtOAc in petroleum ether) to afford the title compound (**5**) as a light yellow solid (135 mg, 10% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.28 (s, 1H), 8.15 (d, J = 2 Hz, 1H), 8.08 (d, J = 9 Hz, 1H), 7.85 (dd, J = 9, 2 Hz, 1H), 7.47 (d, J = 9 Hz, 2H), 7.27 (d, J = 9 Hz, 2H), 4.24 (q, J = 8 Hz, 2H); ^{19}F NMR (282 MHz, DMSO-d_6): δ -72.1 (s, 6F), −74.2 (m, 3F). MS ESI (m/z): [M-H]$^-$ calcd for $C_{17}H_9Br_2F_9NO_3S$, 638.1; found, 638 and 640 (dibromo pattern).

2,4-dibromo-*N*-(4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl)-*N*-(2,2,2-trifluoroethyl)benzenesulfonamide (6)

2,4-dibromo-*N*-(4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl) benzenesulfonamide (**4**) (249 mg, 0.45 mmol), K_2CO_3 (68 mg, 0.49 mmol), and acetonitrile (1.5 mL) were combined and stirred at 23°C. 2,2,2-trifluoroethyl trifluoromethanesulfonate (109 mg, 0.47 mmol) was added, and the reaction was stirred at reflux for 16 h. The reaction was quenched with saturated aqueous NH_4Cl (50 mL) and extracted with dichloromethane (3 × 50 mL). The organic fractions were combined, rinsed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (SiO_2, 0–50% EtOAc in heptane) to afford the title compound (**6**) as a white solid (95 mg, 33% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.78 (s, 1H), 8.17 (s, 1H), 7.80–7.59 (m, 4H), 7.48 (d, J = 9 Hz, 2H), 4.85 (q, J = 9 Hz, 2H); ^{19}F NMR (282 MHz, DMSO-d_6) δ -−71.2 (t, J_{HF} = 9 Hz, 3F), −75.5 (s, 6F). MS ESI (m/z): [M-H]$^-$ calcd for $C_{17}H_9Br_2F_9NO_3S$, 638.1; found, 638 and 640 (dibromo pattern).

2,4-ditritium-*N*-(4-(1,1,1,3,3,3-hexafluoro-2-(2,2,2-trifluoroethoxy)propan-2-yl)phenyl)benzenesulfonamide (7)

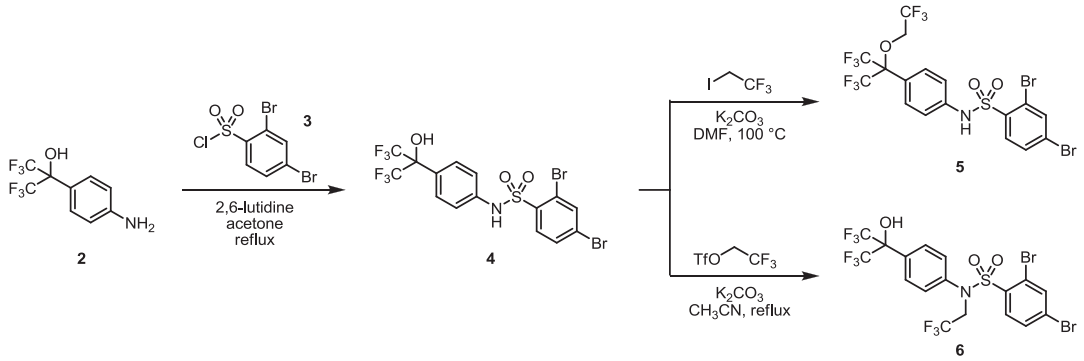
2,4-dibromo-*N*-(4-(1,1,1,3,3,3-hexafluoro-2-(2,2,2-trifluoroethoxy)propan-2-yl)phenyl)benzenesulfonamide (**5**) (7.7 mg, 12.1 μmol) was dissolved in 1% (w/v) methanolic potassium hydroxide (1 mL). The reaction vessel was charged with 10% palladium on carbon (15 mg), and the resultant black mixture was stirred for 3 h at 23°C under an atmosphere of tritium gas (2 Ci, 1 atm). The reaction was then filtered, and the labile tritium was removed under consecutive rotary evaporations from methanol (3 × 5 mL). The crude material was purified by preparatory HPLC (Hichrom Ltd. Ultrasphere C18 ODS 25 × 1 cm column, 5 μm particle size) using a 60–100% gradient of water/acetonitrile (3 mL/min flow rate) over a 60 min period (230 nm UV detection). The relevant fractions were pooled and dried via rotary evaporation to provide the title compound (**7**). The resulting product was re-constituted in ethanol (1 mCi/mL). The radiochemical purity (95.0%) was determined by analytical HPLC (Vydac Genesis C8 150 × 4.6 mm column, 5 μm particle size) using a 40–90% gradient of 20 mM aqueous ammonium acetate and acetonitrile (1 mL/min flow rate) over a 15 min period, and held for an additional 5 min (254 nm UV detection). Specific activity was determined by mass spectroscopy (54 Ci/mmol, ESI (m/z): [M-H]$^-$ calcd for $C_{17}H_9T_2F_9NO_3S$, 484.3; found, 484.0). ^{19}F NMR (376 MHz, DMSO-d_6): δ −72.2 (s, 6F), -74.1 (m, 3F).

2,4-ditritium-*N*-(4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl)-*N*-(2,2,2-trifluoroethyl)benzenesulfonamide (8)

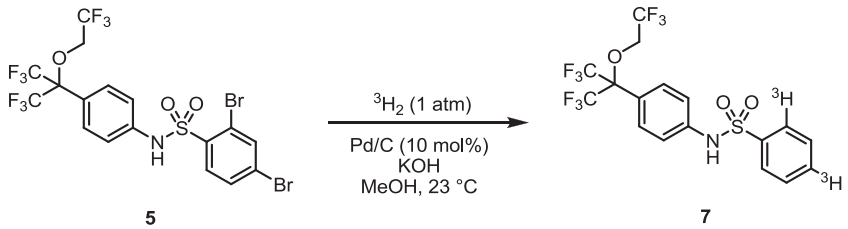
2,4-dibromo-*N*-(4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl)-*N*-(2,2,2-trifluoroethyl)benzenesulfonamide (**6**) (6 mg, 9.4 μmol) was dissolved in 1% (w/v) methanolic potassium hydroxide (1 mL). The reaction vessel was charged with 10% palladium on carbon (15 mg), and the resultant black mixture was stirred for 3 h at 23°C under an atmosphere of tritium gas (2 Ci, 1 atm). The reaction was then filtered, and the labile tritium was removed under consecutive rotary evaporations from methanol (3 × 5 mL). The crude material was purified by preparatory HPLC (Hichrom Ltd. Ultrasphere C18 ODS 25 × 1 cm column, 5 μm particle size) using a 30– 90% gradient of water/acetonitrile (3 mL/min flow rate) over a 60 min period (254 nm UV detection). The relevant fractions were pooled and dried via rotary evaporation to provide the title compound (**8**). The resulting product was re-constituted in ethanol (1 mCi/mL). The radiochemical purity (99.9%) was determined by analytical HPLC (Vydac Genesis C8 150 × 4.6 mm column, 5 μm particle size) using a 40– 90% gradient of 20 mM aqueous ammonium acetate and acetonitrile (1 mL/min flow rate) over a 15 min period, and held for an additional 5 min (254 nm UV detection). Compound **8** also co-chromatographed with compound **1** under the previously mentioned analytical HPLC conditions. Specific activity was determined by mass spectroscopy (48 Ci/mmol, ESI (m/z): [M-H]$^-$ calcd for $C_{17}H_9T_2F_9NO_3S$, 484.3; found, 484.0). ^{3}H NMR (426 MHz, DMSO-d_6): δ 7.78 (s, 1T), 7.76 (s, 1T); ^{19}F NMR (376 MHz, DMSO-d_6): δ −71.0 (m, 3F), −75.4 (s, 6F).

Results and discussion

Synthesis of the target molecule commenced with sulfonamide formation between the commercially available 2-(4-amino-phenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (**2**) and sulfonyl chloride (**3**) under basic conditions (Scheme 1). The resulting sulfonamide (**4**) was subjected to alkylation conditions similar to those described by Li and co-workers for the synthesis of related tertiary sulfonamide analogs.[6] Only one alkylated product was identified in our reaction mixture. Based on the literature precedent from Li and co-workers, we presumed that the alkylation would occur on the sulfonamide nitrogen to provide dibromo-intermediate **6**. After extensive analysis of the alkylation product described in the succeeding texts, we found that the alkylation had instead occurred on the *oxygen* of the hexafluoro-2-isopropanol moiety to provide compound **5**. The *O*-alkylation result was most apparent when compound **5** was subjected to metal-catalyzed tritium-halogen exchange conditions under a tritium gas atmosphere (Scheme 2). The positive and negative ion mass spectrometry data



Scheme 1. Syntheses of dibromo-intermediates **5** and **6**.



Scheme 2. Synthesis of labeled ligand **7**.

of the reaction product matched that of the desired compound, but the HPLC retention time of the labeled product did not match that of T0901317 **1** (Cayman Chemicals). At that point, we were uncertain of the labeled product's chemical structure. To rule out the risk of differing chromatographic retention times due to isotopic fractionation,[7] and to further characterize the labeled product, we explored other analytical methods to gather structural information.

As a standard laboratory practice, labeled products were stored as dispersals in ethanol to minimize radiation exposure risks, increase long-term stability, and ease handling of small sample quantities.[8] Although the ethanolic dispersal had the previously mentioned benefits, it limited our capability to perform ^{1}H NMR analysis of the dispersed labeled product and gather structural information. The amount of labeled product was also too minuscule to acquire a ^{13}C NMR spectrum in a reasonable timeframe, thus we turned our attention toward ^{19}F NMR techniques. The high relative abundance of ^{19}F and compatibility with the ethanolic sample media made ^{19}F NMR a suitable tool for the analysis of the dispersed radioligand.[9] The ^{19}F NMR spectrum of the labeled product had very different fluorine chemical shifts than those for compound **1**, and thus provided additional evidence for the labeled product bearing the structure of compound **7**.[10]

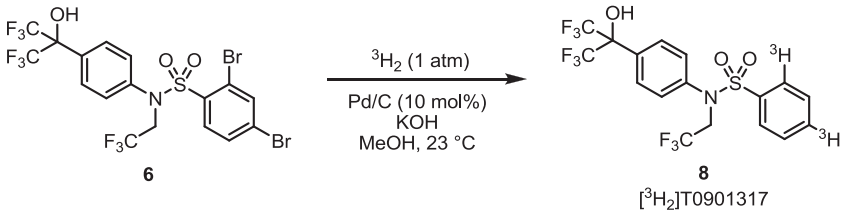
In addition to the comparative ^{19}F NMR studies of compounds **1** and **7**, the connectivity of compound **5** (the precursor to compound **7**) was analyzed using a series of 2D NMR experiments.[11] A ^{1}H—^{15}N HSQC NMR experiment on compound **5** demonstrated the presence of an N—H bond on the sulfonamide. A separate ^{1}H—^{13}C HMBC NMR experiment illustrated that the carbon bearing the bis(trifluoromethyl) moiety was in close proximity to the methylene protons of the 2,2,2-trifluoroethyl group. The outcomes of both 2D NMR experiments were consistent with the structure of compound **5** as drawn (Scheme 1).

A more effective alkylation strategy of compound **4** was envisioned in which the *N*-alkyl product would predominate to provide compound **6** (Scheme 1). A comparison of the reported relative pK_a values of the N—H and O—H hydrogen atoms found in the functional group substructures of compound **4** revealed that they had similar values (pK_a ~ 11).[12,13] Thus, discriminating between the two heteroatoms during the alkylation step could not be achieved on the basis of pK_a alone.

Alteration of the sulfonamide **4** alkylation conditions to include a solvent with greater ionizing-power,[14] and a more reactive electrophile,[15] resulted in the alkylation of the sulfonamide nitrogen and provided the dibromo-intermediate **6** (Scheme 1). This was the only alkylation product in the reaction mixture. Analysis of the ^{19}F NMR spectrum revealed that the fluorine shifts of compound **6** were in close agreement with those of T0901317 (**1**), providing additional confirmation for the structure of compound **6** as drawn (Scheme 1).

Compound **6** was then subjected to a palladium on carbon suspension in methanolic potassium hydroxide under an atmosphere of tritium gas to provide [$^{3}H_2$]T0901317 (Scheme 3, Compound **8**). Compound **8** was manipulated and stored as an ethanolic dispersal (1 mCi/mL). The HPLC retention time and ^{19}F NMR spectrum of the labeled material (**8**) were nearly identical to that of T0901317 (**1**). Tritium NMR analysis indicated the presence of two distinct aromatic tritium atoms on compound **8** in a 1:1 ratio. The radiochemical purity of compound **8** was >99%, and the specific activity was 48 Ci/mmol.

Radioligands **7** and **8** were tested in SPA format to assess their ability to bind to LXRα (Table 1). Compound **7** showed low SB to LXRα, with an average of 19% (±14%) SB across all time points. On the other hand, [$^{3}H_2$]T0901317 (**8**) showed 2.6-fold higher SB to LXRα than ligand **7**, with an average over all time points of 50% (±11%) SB. These SPA binding data were consistent with the ^{19}F NMR characterization of ligand **8** being the desired [$^{3}H_2$] T0901317 product.



Scheme 3. Synthesis of [$^3H_2$]T0901317 (**8**).

Table 1. Specific binding of radioligands **7** and **8** to the LXRα ligand binding domain as assessed in a scintillation proximity assay.

Compound	LXRα (%) specific binding
7	19 (±14%)
8	50 (±11%)

Acknowledgements

We thank Joe Pease for helpful discussions regarding the analytical data.

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] J. R. Schultz, H. Tu, A. Luk, J. J. Repa, J. C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D. J. Mangelsdorf, K. D. Lustig, B. Shan, *Genes Dev.* **2000**, *14*, 2831–2838.
[2] B. Hu, M. Collini, R. Unwalla, C. Miller, R. Singhaus, E. Quinet, D. Savio, A. Halpern, M. Basso, J. Keith, V. Clerin, L. Chen, C. Resmini, Q.-Y. Liu, I. Feingold, C. Huselton, F. Azam, M. Farnegardh, C. Enroth, T. Bonn, A. Goos-Nilsson, A. Wilhelmsson, P. Nambi, J. Wrobel, *J. Med. Chem.* **2006**, *49*, 6151–6154.
[3] N. Mitro, L. Vargas, R. Romeo, A. Koder, E. Saez, *FEBS Lett.* **2007**, *581*, 1721–1726.
[4] N. Kumar, B. Lyda, M. R. Chang, J. L. Lauer, L. A. Solt, T. P. Burris, T. M. Kamenecka, P. R. Griffin, *ACS Chem. Biol.* **2012**, *7*, 672–677.
[5] B. A. Janowski, M. J. Grogan, S. A. Jones, G. B. Wisely, S. A. Kliewer, E. J. Corey, D. J. Mangelsdorf, *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 266–271.
[6] L. Li, J. Liu, L. Zhu, S. Cutler, H. Hasegawa, B. Shan, J. C. Medina, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1638–1642.
[7] C. N. Filer, *J. Labelled Comp. Radiopharm.* **1999**, *42*, 169–197.
[8] R. Voges, J. R. Heys, T. Moenius, Introduction. Preparation of Compounds Labeled with Tritium and Carbon-14, 1st ed., John Wiley & Sons, Ltd., West Sussex, UK, **2009**, pp 6–15.
[9] W. R. Dolbier, An overview of fluorine NMR. Guide to Fluorine NMR for Organic Chemists, 1st ed., John Wiley & Sons, Inc., Hoboken, NJ, **2009**, pp 9–34.
[10] NMR spectra for the T0901317 samples supplied by Cayman Chemicals and Sigma-Aldrich were identical: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.81 (s, 1H), 7.74 (t, J = 6 Hz, 1H), 7.67-7.56 (m, 6H), 7.29 (d, J = 8 Hz, 2H), 4.63 (q, J = 8 Hz, 2H); ^{19}F NMR (282 MHz, DMSO-d_6): δ -71.5 (t, J_{HF} = 14 Hz, 3F), -75.4 (s, 6F).
[11] S. Berger, S. Braun, 2D NMR spectroscopy with field gradients. 200 and More NMR Experiments: A Practical Course, 2nd ed., Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, **2004**, pp 525–612.
[12] S. M. Ivanova, B. G. Nolan, Y. Kobayashi, S. M. Miller, O. P. Anderson, S. H. Strauss, *Chem. Eur. J.* **2001**, *7*, 503–510.
[13] F. G. Bordwell, D. Algrim, *J. Org. Chem.* **1976**, *41*, 2507–2508.
[14] J. March, Aliphatic nucleophilic substitution. Advanced Organic Chemistry: Reactions Mechanism and Structure, 4th ed., John Wiley & Sons, Inc., New York, NY, **1992**, pp 357–362.
[15] J. March, Aliphatic nucleophilic substitution. Advanced Organic Chemistry: Reactions Mechanism and Structure, 4th ed., John Wiley & Sons, Inc., New York, NY, **1992**, pp 352–354.