Pergamon

Tetrahedron Letters, Vol. 35, No. 51, pp. 9521-9524, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)02140-6

Synthesis of [^{35}S]3*H*-1,2-Benzodithiole-3-one-1,1-Dioxide: Application in the Preparation of Site-specifically ^{35}S-labeled Oligonucleotides.

Radhakrishnan P. Iyer, Weitian Tan, Dong Yu and Sudhir Agrawal
Hybridon Inc., One Innovation Drive, Worcester, MA 01605

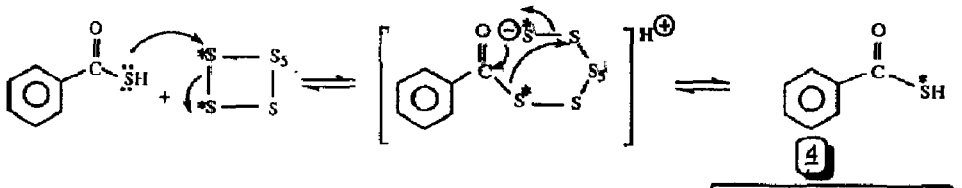
Abstract: *The synthesis of [^{35}S]3H-1,2-benzodithiole-3-one-1,1-dioxide (1) and its use in the preparation of site-specifically ^{35}S-labeled oligonucleotides is illustrated.*

The use of 3*H*-1,2-benzodithiole-3-one-1,1-dioxide (**1**) as a sulfurizing reagent,[1a,b] in conjunction with phosphoramidite chemistry,[2a,b] is well established for the routine syntheses and manufacture of a variety of oligonucleoside phosphorothioates (PS-oligos).[3a-c] Concurrently, for biological studies, ^{35}S-labeled PS-oligos are prepared using H-phosphonate chemistry.[4a,b] However, with H-phosphonate chemistry, it is difficult to achieve site-specific labeling of PS-oligos and to carry out preparation of ^{35}S-labeled oligonucleoside phosphorothioate constructs[4b] such as those with (a) mixed PO-PS backbones (b) heterogeneous backbones eg., phosphorothioate-methyl phosphonate ("chimeric oligos") and (c) mixed ribonucleotide-deoxyribonucleotide population ("hybrid oligos"). In connection with our *in vivo* pharmacokinetic studies of "antisense" PS oligos,[5] we needed site-specifically ^{35}S-labeled chimeric and hybrid PS oligos. Reported herein is the synthesis of [^{35}S]3*H*-1,2-benzodithiole-3-one-1,1-dioxide[4c] and its use in the preparation of a variety of ^{35}S-labeled oligonucleotides.

In order to access [^{35}S]**1**, we needed ^{35}S-labeled thiolacetic acid whose preparation was attempted using a high temperature (125 °C) exchange reaction between thiolacetic acid and [^{35}S].[6] However the high volatility (b.p. 81 °C) and vapor pressure (v.p.) of thiolacetic acid posed problems when using ^{35}S with high specific activity (32 mCi/μmol). To circumvent this difficulty, the commercially available thiobenzoic acid (**4**), a thiol acid with a higher b.p. and lower v.p. was chosen. We validated the use of **4** in the synthesis of **1**, by preparing **2**, the precursor to **1** (Scheme 2). Presumably, **2** is formed[7] *via* the intermediate **3** and longer time (4h) was required for completion of the reaction.[8a] A crystallized sample of **2**, thus synthesized, was identical in all respects (m.p., ^{1}H-NMR and ^{13}C-NMR) with that obtained by the reported procedure.[1a-b] Having demonstrated the feasibility of using **4** in the preparation of **2**, [^{35}S]**4** was conveniently prepared (Scheme 1) in good radiochemical yields which was then converted to [^{35}S]**2** (Scheme 2). Upon careful oxidation with H_2O_2 in trifluoroacetic acid[1a-b, 8b] [^{35}S]**2** gave [^{35}S]**1** as a white crystalline solid in ca. 30% chemical yield (based on **9**)[8b] (specific activity 91 μCi/μmol). For the oxidative sulfurization reaction in oligonucleotide synthesis, a solution of [^{35}S]**1** (1% in anhydrous acetonitrile) was used (Scheme 3).

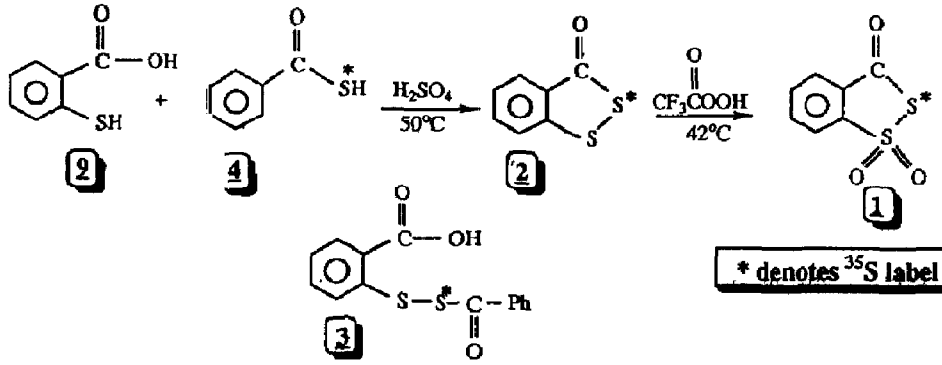
To demonstrate the use of [^{35}S]**1** in site-specific labelling, we prepared ^{35}S-d[TpsT] on a 0.1 μmol scale, in an automated DNA synthesizer (Biosearch) using phosphoramidite chemistry.[2a,b] To incorporate the ^{35}S label, the synthesis cycle was interrupted after the formation of the internucleotidic *phosphite* linkage, the CPG was removed from the column and treated with a stock solution of [^{35}S]**1**(*vide infra*) (15 μL, 30 min) followed by a solution of "cold" **1** (2% in acetonitrile, 100 μL, 10 min).[8c] Following synthesis, the dimer was isolated by treatment of the CPG with aqueous NH_4OH (30%, 2 h, 55 °C). When examined by

Scheme 1: Preparation of ^{35}S-labeled thiobenzoic acid (4)



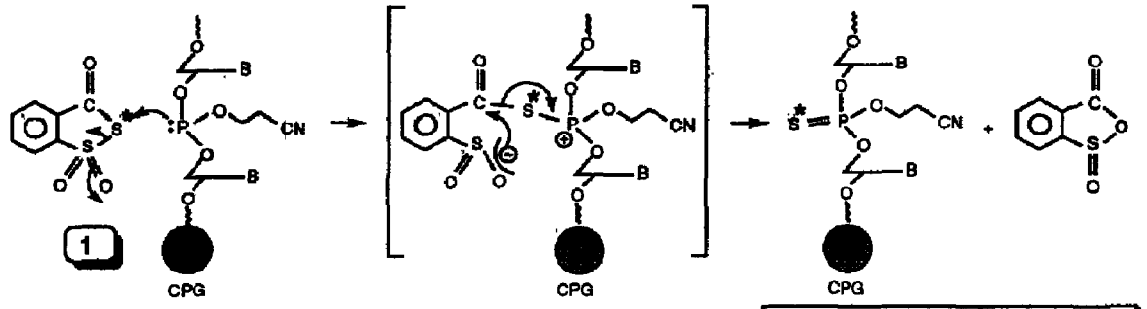
*** denotes ^{35}S label**

Scheme 2: Preparation of ^{35}S-labeled sulfurizing reagent 1



*** denotes ^{35}S label**

Scheme 3: Incorporation of site-specific ^{35}S- label in an oligonucleotide using 1



B = Base; * denotes ^{35}S - label

polyacrylamide gel electrophoresis (PAGE, 20%), the autoradiographic image of the dimer was superimposable on its UV-shadowed band (data not shown). In reverse-phase HPLC[8d] using UV detection interfaced with flow-scintillation analysis, radioactivity profile of the dimer was superimposable on its UV-absorbing peaks with retention times [^{35}S]R_p-d[TpsT] (Rt 22.7 min) and [^{35}S]S_p-d[TpsT] (Rt 24.0 min)(R_p:S_p, 60:40) (Fig. 1). We then prepared a variety of oligonucleotides 5-8 (1 μ mol scale) bearing a pre-determined site of incorporation of the ^{35}S-label (Fig. 2) using procedures as described for the dimer. The oxidative-sulfurization was quantitative as determined by "trityl assays." After standard deprotection, the

crude oligonucleotides 5-8 were isolated by preparative PAGE (20%), desalted on a Sephadex G-25 column and examined by analytical PAGE (20%) (Fig. 3) and ion-exchange HPLC[8d,e] (Fig. 4). The oligonucleotides 5-8 thus obtained had a specific activity ca. 23 ~ 25 μCi/μmol.

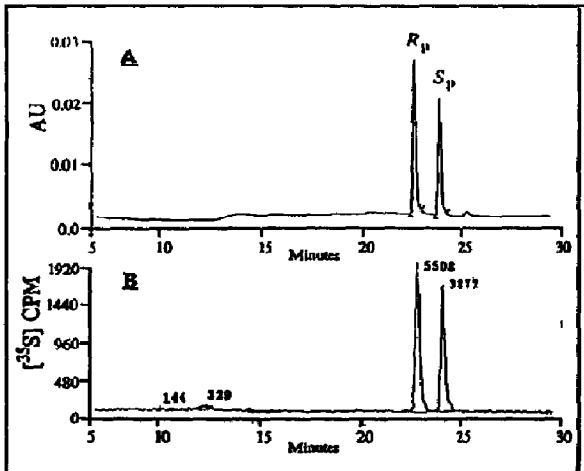


Fig. 1. HPLC (reverse-phase) profile[8d] of [^{35}S]R_p-d[TpsT] and [^{35}S]S_p-d[TpsT]. **Panel A**, by UV detection at λ 260 nm; **Panel B**, by Flow Scintillation Analysis.[8d]

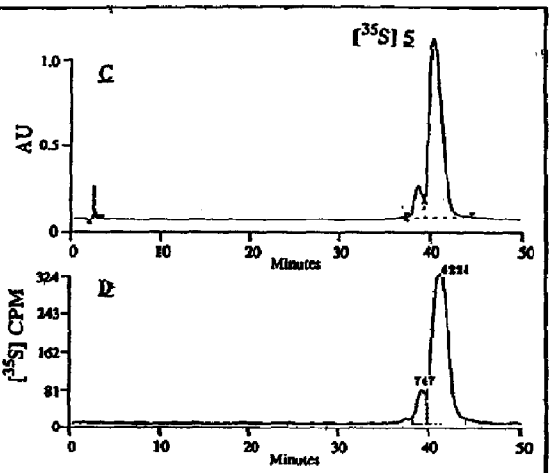


Fig. 4. HPLC (ion-exchange) profile[8e] of [^{35}S]5. **Panel C**, by UV detection at λ 260 nm; **Panel D**, by Flow Scintillation Analysis.[8d]

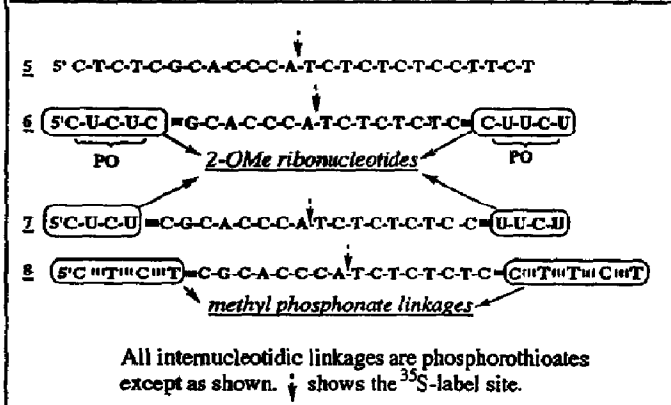


Fig. 2. Oligonucleotide sequences 5-8.

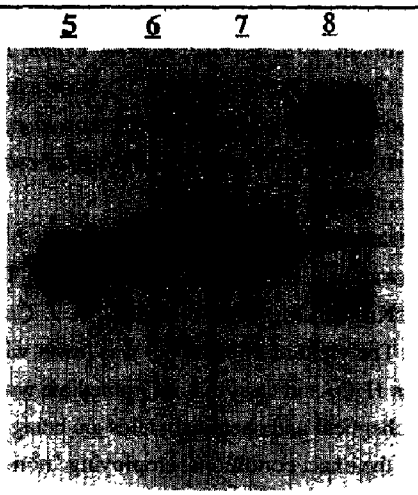


Fig. 3. Autoradiography of oligonucleotides 5-7 (purified), and 8 (crude) subjected to PAGE.

Typical experimental procedures: *a) Synthesis of [^{35}S]2,* In an eppendorf tube (1.5 mL) was placed 4 (6.5 μL, 55 μmol) and [^{35}S] (5mCi, 1Ci/mg atom in 100 μL of toluene) and the contents heated at 97 °C for 5 h. The solution was evaporated to dryness under argon, and thiosalicylic acid (9) (5 mg) and sulfuric acid (98%, 50 μL) were added at 0 °C. After heating at 50 °C for 3h, the brown reaction mixture was cooled to -78 °C and quenched with water (600 μL). The solution was warmed, transferred to a vial and diluted with 500 μL water. The solution was extracted with methylene chloride (4 X 3 mL) and the organic

layer washed with Na_2CO_3 (5%, 2 X 2 mL). The organic layer was evaporated to dryness and the resulting solid was dissolved in warm hexane (2 mL) and the solution was evaporated to dryness under argon to give a yellow solid (4 mg, 2.17mCi, 44% yield). This material was used as such in the next step or stored at -20 °C until ready to use. ***b) Synthesis of [^{35}S]1.*** To 4 mg of **2** in an eppendorf tube, cooled to 0 °C, was added trifluroacetic acid (25 µL) and 30% H_2O_2 (12 µL). The reaction mixture was kept at 42 °C for about 2 h (as monitored by TLC, silica gel, chloroform),[1b] and then was quenched with ice-cold water (300 µL). The resulting white precipitate was washed with water (2 X 300 µL) and dried *in vacuo* to give [^{35}S]**1** (2 mg, total activity of 915 µCi, specific activity 91 µCi/µmol). A stock solution of [^{35}S]**1** in anhydrous acetonitrile (2mg, 91 µCi/µ mol in 200 µL acetonitrile) could be stored at -20 °C until ready to use for the oxidative sulfurization reaction.

In view of the ready availability of [^{35}S]**1** and its use in the preparation of site-specifically ^{35}S-labeled oligonucleotides, several biochemical and biomedical applications of [^{35}S]**1** can be anticipated.[9]

References and notes:

1. (a) Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1253-54. (b) Iyer, R. P.; Phillips, L. R.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Org. Chem.* **1990**, *55*, 4693-98.

2. (a) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859-1862. (b) For a review see Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223-2311.

3. (a) Padmapriya, A.; Tang, J-Y.; Agrawal, S. *Antisense Res.& Dev.* **1994**, *4*, 185-199. (b) Padmapriya, A.; Agrawal, S. *Bioorg. & Med. Chem. Lett.* **1993**, *3*, 761-764. (c) Andrad, M.; Scozzari, A.; Cole, D. L.; Ravikumar, V. T. *Bioorg. & Med. Chem. Lett.* **1994**, 2017-2022.

4. (a) Garegg, P. J.; Regberg, T.; Stawinski, J.; Stromberg, R. *Chem. Scr.* **1985**, *25*, 280-82. (b) *In order to ensure stereochemically identical product, it is desirable to use the same "chemistry" and sulfurizing reagent both for synthesis and biological evaluation.* (c) Disadvantages of sulfurization using elemental sulfur has been alluded to earlier.[1a,b]

5. Agrawal, S.; Tang, J-Y.; Temsamani. J. *Proc. Natl. Acad. Sci. (USA)* **1991**, *88*, 7595-7599.

6. Kawamura, S.; Sato, A.; Nakabayashi, T.; Hamada, M. *Chemistry Lett.* **1975**, 1231-1234.

7. McKibben, M.; McClelland E. W. *J. Chem. Soc.* **1923**, 170-173.

8. (a) The yield of **2** (ca. 60%) was lower with **4** than that (82%) with thiolacetic acid.[1a,b] (b) Avoiding excess H_2O_2 and carrying out immediate work-up after completion of reaction, give good yields of [^{35}S]**1**; radiochemical and chemical yields are being optimized further. (c) A sample of the dimer (TpsT) prepared under the exact conditions, employing "non-radioactive" **1**, revealed that the conversion to TpsT was > 99% (data not shown). (d) All HPLC analyses were done on a Waters 660E instrument with a 996 Photodiode Array UV detector interfaced with a Radiomatic 500 TR v3.00 Flow Scintillation Analyzer (Packard); reverse-phase HPLC was done using a 8 NV C_{18} 4µ Radial Pak cartridge column (8 X 100 mm), gradient (100% A to 60% B over 60 minutes) of buffer A (0.1M $CH_3CO_2NH_4$) and buffer B (80:20, CH_3CN:0.1 M $CH_3CO_2NH_4$), flow rate 1.5 mL/min. (e) Ion-exchange HPLC was done using a GEN-PAK FAX column (4.6 X 100 mm) at 65 °C using a gradient (80% A to 100% B over 50 min) of Buffer A (25 mM Tris HCl, pH 8.5, 10% CH_3CN) to Buffer B (25 mM Tris HCl, 2 M LiCl, pH 8.5, 10% CH_3CN), flow rate 0.5 mL/min.

9. *This paper is dedicated to the late Palghat T. S. Mani Iyer.*

(*Received in USA* 28 *September* 1994; *revised* 24 *October* 1994; *accepted* 27 *October* 1994)