ORGANIC LETTERS

2006
Vol. 8, No. 19
4243−4246

An Unsymmetrical Approach to the Synthesis of Bismethylene Triphosphate Analogues

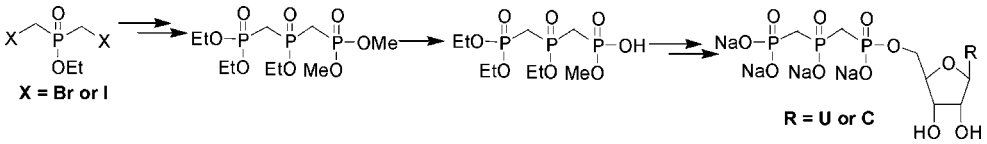
Scott D. Taylor,*,† Farzad Mirzaei,† and Stephen L. Bearne‡

Department of Chemistry, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, Canada, N2L 3G1, and Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 1X5

s5taylor@sciborg.uwaterloo.ca

Received June 22, 2006

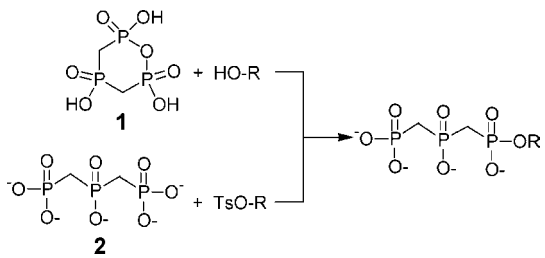
ABSTRACT



A protected, unsymmetrical bismethylene triphosphate analogue was prepared by sequential Michaelis–Arbuzov reactions on ethyl bis(halomethyl)phosphinates. This species was monodeprotected at one of the terminal phosphonate groups in high yield. The resulting monodeprotected compound was used to achieve the first syntheses of the bismethylene triphosphate analogues of UTP and CTP.

Polyphosphorylated compounds, such as nucleotides, coenzymes, vitamins, and isoprenoids, are widespread in nature and have key roles in numerous biological functions. Nonhydrolyzable polyphosphate analogues have been used extensively as probes and inhibitors of enzymes and other proteins that bind or hydrolyze polyphosphates.[1] The methylene group figures prominently among the various groups that have been used to replace the labile pyrophosphate oxygens. Although methylene analogues of diphosphates are readily constructed, the synthesis of bismethylene triphosphate (BMT) analogues still represents a considerable challenge.[2] BMT derivatives are usually prepared by reacting a nucleophile with trimetaphosphate **1**[3] or, more commonly, by reacting alkylammonium salts of **2** with tosyl ester acceptors[1e−h,4a,b] (Scheme 1). However, these reactions proceed in very low yields and usually require a lengthy and difficult purification. An alternative approach is to couple the monoacid of a protected BMT of type **3** to an acceptor (Scheme 2). The advantage of this route is that the purification issues encountered using the approaches outlined in

Scheme 1



† University of Waterloo.
‡ Dalhousie University.

(1) For some examples see: (a) Biller, S.; Forster, C. *Tetrahedron* **1990**, *46*, 6645−6658. (b) Blackburn, G. M.; Langston, S. P. *Tetrahedron Lett.* **1991**, *32*, 6425−6428. (c) Morr, M.; Wray, V. *Angew. Chem., Int. Ed. Eng.* **1994**, *33*, 1394−1396. (d) Yanachkov, I.; Pan, J. Y.; Wessling-Resnick, M.; Wright, G. E. *Mol. Pharmacol.* **1997**, *51*, 47−51. (e) Ma, Q.-F.; Kenyon, G. L.; Markham, G. D. *Biochemistry* **1990**, *29*, 1412. (f) Dalton, A.; Hornby, D. P.; Langston, S. P.; Blackburn, G. M. *Biochem. J.* **1992**, *287*, 871−879. (g) Labataille, P.; Pelicano, H.; Maury, G.; Imbach, J.-L.; Gosselin, G. *Biooorg. Med. Chem. Lett.* **1995**, *5*, 2315−2320. (h) Spelta, V.; Mekhalfia, A.; Rejman, D.; Thompson, M.; Blackburn, G. M.; North, R. A. *Br. J. Pharmacol.* **2003**, *140*, 1027−1034.

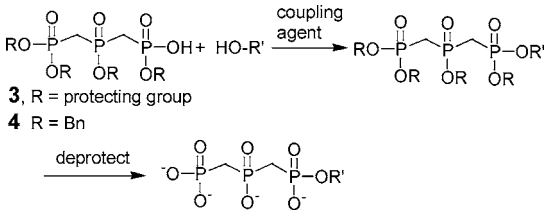
(2) Klein, E.; Mons, S.; Valleix, A.; Mioskowski, M.; Lebeau, L. *J. Org. Chem.* **2002**, *67*, 146−153 and references therein.

(3) Towbridge, D. B.; Yamamoto, D. M.; Kenyon, G. L. *J. Am. Chem. Soc.* **1972**, *94*, 3816.

(4) (a) Stock, J. A. *J. Org. Chem.* **1979**, *44*, 3997−4000. (b) Blackburn, G. M.; Guo, M. J.; Langston, S. P.; Taylor, G. E. *Tetrahedron Lett.* **1990**, *31*, 5637−5640.

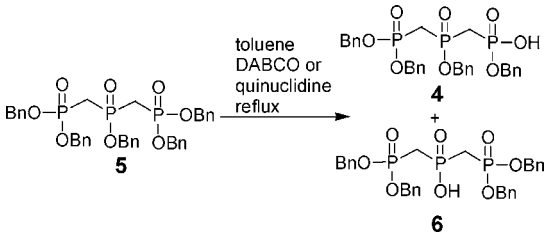
10.1021/ol0615432 CCC: $33.50
Published on Web 08/18/2006

Scheme 2



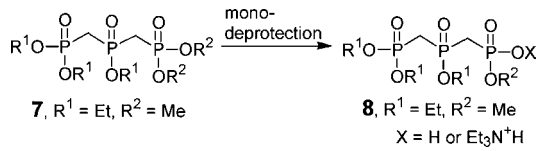
Scheme 1 are for the most part overcome since purification of the coupled product is readily accomplished with flash chromatography. The final BMT derivative is obtained after deprotection of the coupled product under mild conditions. This route, pioneered by Mioskowski and co-workers, has been used to prepare BMT analogues of AZT,[5] thiamine triphosphate,[6] and Ap_3A and Gp_3A.[2] The AZT analogue was recently used to obtain antibodies against AZT triphosphate.[7] The tetrabenzyl derivative **4** was used for these studies and the benzyl groups were removed at the end of the syntheses with TMSBr[5,6] or by hydrogenolysis.[1] Although this route appears attractive, the synthesis of compound **4** and its precursor, symmetrical pentabenzyl ester **5** (Scheme 3), has

Scheme 3



been fraught with difficulties which limits the appeal of this approach. For example, Saady et al. reported that **5** could be selectively monodeprotected at one of the terminal phosphonate moieties in 95−97% yield with 1 equiv of DABCO or quinuclidine in refluxing toluene (Scheme 3).[8] However, it was later reported that this reaction gives a mixture of **4** and **6** that could not be separated.[5,6] Consequently, a mixture of **4** and **6** was used to prepare BMT's of thiamine and AZT.[5,6] After complete deprotection of the coupled products the resulting regioisomers were separated by HPLC. This approach would be considerably improved if a highly selective monodeprotection of one of the terminal phosphonates of compound **5** could be achieved. If this is not possible, then an alternative would be to prepare an unsymmetrical BMT ($R^1 \neq R^2$ in Scheme 4) since this would

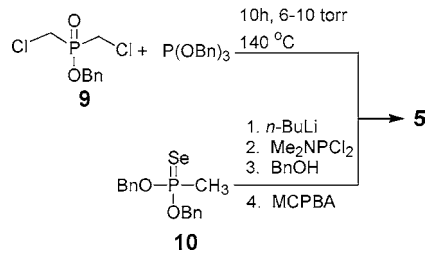
Scheme 4



allow for the selective cleavage of one of the terminal esters. Methyl and ethyl protecting groups should be applicable since TMSBr can also readily remove such esters. Here we report a straightforward synthesis of **5** and our attempts to achieve a selective deprotection of one of its terminal phosphonate moieties. We also report the synthesis of the unsymmetrical BMT **7** via sequential Michaelis−Arbuzov reactions on bishalomethylene phosphinates. A highly selective deprotection of the terminal methylphosphonate moiety in **7** yielded monodeprotected **8** that was used for the first synthesis of the BMT analogues of UTP and CTP.

Due to the conflicting reports on the monodeprotection of **5**, we initiated our studies by preparing **5** and determining if a selective monodeprotection of one its terminal benzyl groups could be achieved. Saady et al. reported that benzyl derivative **5** could be prepared in a 71% yield by a Michaelis−Arbuzov reaction between benzyl bischloromethylphosphinate (**9**) with 4 equiv of tribenzyl phosphite (TBP) at 6−10 Torr at 140 °C (Scheme 5).[9] However, Mons

Scheme 5



et al. stated that the synthesis of **5** using this approach is difficult to reproduce.[10] This is due to a competing reaction of the in situ generated benzyl chloride with TBP. The TBP is rapidly consumed by reaction with benzyl chloride unless the benzyl chloride is efficiently removed from the reaction mixture as it is formed.[9] Consequently, these workers reported an alternative approach to **5** by first preparing selenophosphonate **10** and then reacting the anion of **10** with *N*,*N*-dimethylphosphonamidous dichloride, followed by reaction with benzyl alcohol and then oxidation with MCPBA (Scheme 5). A yield of 37% was reported over these three steps.[2,10]

(5) Brossette, T.; Le Faou, A.; Goujon, L.; Valleix, A.; Creminon, C.; Grassi, J.; Mioskowski, C.; Lebeau, L. *J. Org. Chem.* **1999**, *64*, 5083−5090.

(6) Klein, E.; Nghiem, H.-O.; Valliex, A.; Mioskowski, C.; Lebeau, L. *Chem. Eur. J.* **2002**, *8*, 4649−4655.

(7) Becher, F.; Schlemmer, D.; Pruvost, A.; Nevers, M.-C.; Goujard, S. J.; Guerreiro, C.; Brossette, T.; Lebeau, L.; Creminon, C.; Grassi, J.; Benech, H. *Anal. Chem.* **2002**, *74*, 4220−4227.

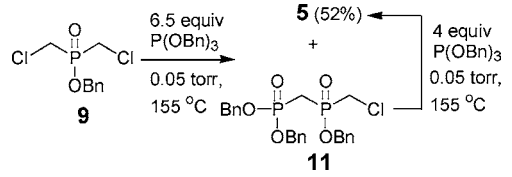
(8) Saady, M.; Lebeau, L.; Mioskowski, C. *J. Org. Chem.* **1995**, *60*, 2946−2947.

(9) Saady, M.; Lebeau, L.; Mioskowski, C. *Helv. Chim. Acta* **1995**, *78*, 670−678.

(10) Mons, S.; Klein, E.; Mioskowski, C.; Lebeau, L. *Tetrahedron Lett.* **2001**, *2*, 5439−5442.

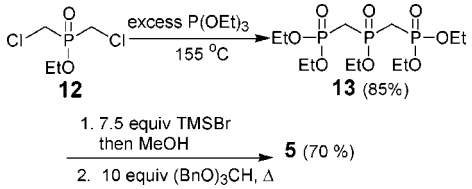
We initially examined the selenophosphonate route[2,10] to prepare **5**. However, in our hands, this procedure gave **5** in yields <10%. Consequently, we decided to examine Saady et al.'s original approach using tribenzyl phosphite and phosphinate **9**.[9] Using the conditions reported by Saady et al.[9] mentioned earlier only trace amounts of **5** were obtained. Nevertheless, it was found that by reacting **9** in the presence of 6.5 equiv of tribenzyl phosphite at 155 °C and 0.05 Torr for 16 h, a mixture of **5**, **11**, and dibenzyl benzylphosphonate could be obtained, which was separated by careful chromatography (Scheme 6).[11] Phosphinate **11** was then reacted

Scheme 6



again in a similar manner with 4 equiv of tribenzyl phosphite to give **5** in an overall 52% yield (Scheme 6). Although this procedure yielded **5**, it was tedious and the separation of **5** from dibenzyl benzylphosphonate and **11** required several columns. Consequently, an alternative route to **5** was developed (Scheme 7). Ethyl derivative **13** was readily

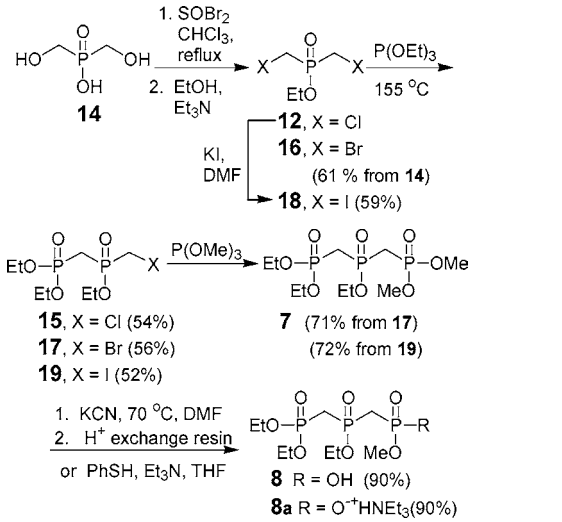
Scheme 7



prepared in 85% yield by reaction of phosphinate **12** with triethyl phosphite (TEP).[12a,b] Reaction of **13** with 7.5 equiv of TMSBr followed by treatment with MeOH and then reaction of the resulting crude acid with 10 equiv of tribenzylorthoformate at 150 °C for 3 h gave compound **5** in a 70% yield (from compound **13**).[13] Purification by column chromatography was straightforward and compound **5** was consistently obtained in good yield even on a multigram scale. The selective removal of a terminal benzyl group in **5** was first attempted with the conditions described by Saady et al. (1.0 equiv of DABCO or quinuclidine in toluene, reflux 2 h).[8] However, in our hands, this gave a complex mixture of products. After some experimentation, we found that a mixture of **4** and **6** in a 4:1 ratio could be obtained in a 67% yield by reacting **5** with 1.0 equiv of DABCO in benzene at 60 °C for 6 h. We also attempted the selective removal of one of the terminal benzyl groups using a variety of nucleophiles (NaI, LiBr, thiols, amines) in a variety of solvents; however, all of these procedures gave inseparable mixtures of **4** and **6**, which is consistent with the results of Brossette et al. and Klein et al.[5,6] Consequently, we decided to prepare unsymmetrical BMT **7**.

Our approach to **7** was by sequential Michaelis−Arbuzov reactions on ethyl bis(halomethyl)phosphinates (Scheme 8).

Scheme 8



Compound **15** was easily prepared in a 54% yield with the procedure of Medved et al., which involved reacting **12** with 1.8 equiv of TEP at 175 °C for 7 h.[14] However, attempts to produce unsymmetrical BMT **7** by reaction of **15** with refluxing excess trimethyl phosphite (TMP) were not successful due to the low reactivity of **15** at this temperature.[15] Therefore, bromo and iodo derivatives **16** and **18** were prepared anticipating that these would be more reactive than **15**. Iodo phosphinate **18** was prepared in 59% yield by reacting ester **12** with KI in DMF at 105 °C for 3 h.[16] Bromo derivative **16** has never before been reported. However, it was easily prepared by reacting **14**[17] with thionyl bromide in refluxing $CHCl_3$ for 6−7 h and then reacting the crude phosphinyl bromide with ethanol in the presence of triethylamine (61% yield, 2 steps). Reaction of **16** with 1.8 equiv of TEP at 150 °C for 2 h gave bromo derivative **17** in 56% yield while reaction of **18** with 5 equiv of TEP at 150 °C for 75 min gave iodo derivative **19** in 52% yield. Reaction

(11) The addition of more tribenzyl phosphite to the reaction resulted in an increase in byproducts.

(12) (a) Bel'skii, V. E.; Zyablikova, T. A.; Panteleeva, A. R.; Shermergorn, I. M. *Dokl. Akad. Nauk SSSR* **1967**, *177*, 340−343. (b) Maier, L. *Helv. Chim. Acta* **1969**, *52*, 827−845.

(13) The tetrabenzyl ester of methylene diphosphonate has been prepared with this approach: Gil, L.; Opas, E. E.; Rodan, G. A.; Ruel, R.; Seedor, J. G.; Tyler, P. C.; Young, R. N. *Bioorg. Med. Chem.* **1999**, *7*, 901−919.

(14) Medved, T. Y.; Polikarpov, Y. M.; Pisareva, S. A.; Matrosov, E. I., Kabachnik, M. I. *Izv. Akad. Nauk. USSR, Ser. Khim.* **1968**, *9*, 2062−2070.

(15) We also attempted this reaction with high boiling point solvents such as various xylenes; however, the reaction was very slow and after 24 h only small amounts of the desired products formed.

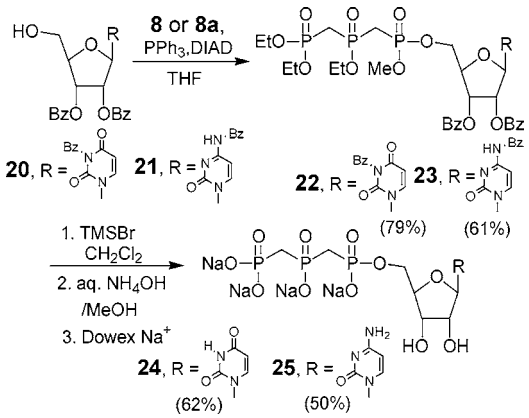
(16) Mukhametzyanova, E. K.; Panteleeva, A. R.; Shermergorn, I. M. *Izv. Akad. Nauk. SSSR, Ser. Khim.* **1967**, *7*, 1597−1598.

(17) Maier, L. *J. Organomet. Chem.* **1979**, *178*, 157−169.

of iodo derivative **19** with excess TMP at 125 °C gave compound **7** in a 72% yield. However, this reaction was tedious to perform since the methyl iodide produced in the reaction rapidly converted all of the TMP to dimethyl methylphosphonate well before the reaction was complete. Addition of more TMP resulted in an increase in byproducts. To obtain reasonable yields, the reaction had to be monitored by ^{31}P NMR and when the TMP was consumed the reaction was cooled to room temperature and the dimethyl methylphosphonate removed by high vacuum rotary evaporation. More TMP was then added and the reaction continued and this process was repeated until all of **19** was consumed. The reaction of bromo derivative **17** with excess TMP at 130 °C gave a similar yield of compound **7**. However, dimethyl methylphosphonate was produced at a much lower rate and the reaction was much easier to perform. Selective deprotection of **7** was achieved by reaction with 1.0 equiv of KCN in DMF at 70 °C for 5 h followed by conversion of the potassium salt to the free acid by passage through a Dowex H^+ ion exchange column. This gave acid **8** in 90% yield. Alternatively, **8** could be obtained in a 90% yield as its triethylammonium salt (**8a**) by reacting **7** with 1.2 equiv of PhSH and 3 equiv of Et_3N in THF at room temperature for 24 h.

To determine if compound **8** or **8a** could be used in the synthesis of BMT's of biomolecules, **8** was coupled to 2′,3′-*O*,*N*^{3}-tribenzoyluridine (**20**) via a Mitsunobu reaction to give compound **22** in a 79% yield (Scheme 9). Surprisingly, we failed to obtain coupled product **23** when reacting **8** with 2′,3′-*O*,*N*^{4}-tribenzoylcytidine (**21**) under the same conditions. However, employing the triethylammonium salt **8a** and using modified Mitsunobu conditions[18] the desired coupled product **23** was obtained in 61% yield. Complete deprotection of **22** and **23** was achieved by subjecting them to TMSBr for 24 h followed by treatment with aq NH_4OH-MeOH.[19] The negative electrospray mass spectra of the crude deprotected

Scheme 9



products suggested that some cleavage of the BMT moiety from the nucleoside had occurred, most likely during the TMSBr reaction. Nevertheless, after purification by RP-HPLC and conversion to their sodium salts with a Dowex Na^+ ion-exchange resin, pure uridine and cytidine BMT's **24** and **25** were obtained in 62% and 50% yield, respectively.

In summary, straightforward syntheses of the symmetrical BMT **5** and unsymmetrical BMT **7** were developed. A selective monodeprotection could not be accomplished with **5** but was readily achieved in high yield with **7**. The resulting monodeprotected compounds **8** and **8a** were used to effect the first syntheses of the BMT analogues of UTP (**24**) and CTP (**25**). This approach should be readily applicable to the synthesis of other BMT derivatives of biological interest.

Acknowledgment. This work was supported by a Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery grant to S.D.T. and an NSERC Collaborative Health Research Project Grant to S.L.B. and S.D.T.

Supporting Information Available: Preparation procedures and characterization data for **5**, **7**, **8**, **8a**, **16**, **17**, **19**, and **22**−**25**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0615432

(18) Norbeck, D. W.; Kramer, J. B.; Lartey, P. A. *J. Org. Chem.* **1987**, *52*, 2174−2179.

(19) Brossette et al. have reported that some anomerization occurred after subjecting a benzyl-protected BMT analogue of an AZT derivative to TMSBr (ref 5). However, ^{31}P and ^{1}H NMR analysis of our crude reaction mixtures revealed that anomerization had not taken place. This could be due to participation of the benzoyl protecting group at the 2′ position.