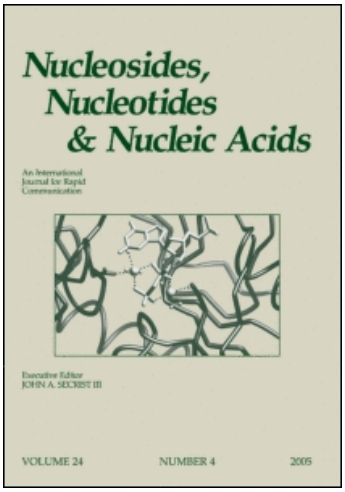

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5-[2-(2-Methoxy)bornyl]tetrazole as Catalyst for Diastereoselective Synthesis of 2'-Deoxy Dinucleoside (3', 5')-Methylphosphonates

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5-[2-(2-METHOXY)BORNYL]TETRAZOLE AS CATALYST FOR DIASTEREOSELECTIVE SYNTHESIS OF 2′-DEOXY DINUCLEOSIDE (3′, 5′)-METHYLPHOSPHONATES

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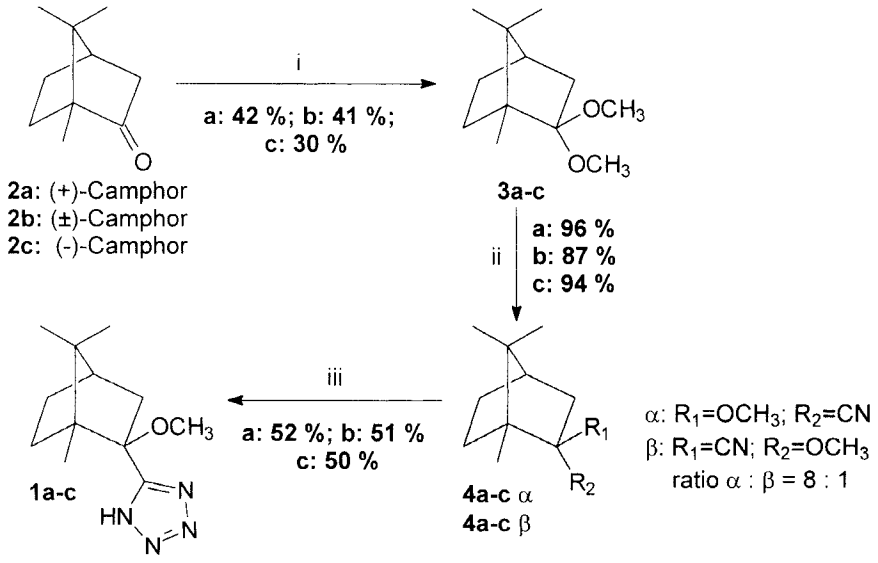
ABSTRACT: The synthesis of 5-[2-(2-methoxy)bornyl]tetrazole **1** is described. **1** is used as catalyst for the diastereoselective synthesis of some 2′-deoxy dinucleoside (3′, 5′)-methylphosphonates in solution.

INTRODUCTION

Oligonucleoside methylphosphonates are well established in the antisense strategy to control gene expression in mammalian cells. They are stable against degradation by cellular nucleases and are taken up intact by cells in culture.[1,2] Due to the chirality at phosphorus oligonucleoside methylphosphonates containing *n* methylphosphonate linkages consist of a mixture of 2^n diastereomers.[3] It has been demonstrated that methylphosphonate oligonucleosides containing linkages with Rp configuration have higher Tm values than the corresponding oligonucleotides containing linkages with Sp configuration.[4,5] Recently, an antisense application of oligonucleotides containing chirally pure Rp methylphosphonates has been reported.[6]

For developing oligonucleoside methylphosphonates into therapeutic agents a simple and convenient method for their stereocontrolled synthesis is necessary. Some methods for this purpose have been summarized[7] or were published recently.[8-11] Most of these methods use diastereomerically pure precursors, which are coupled in a stereoselective or stereospecific manner using P(V) chemistry. We are strongly interested in the phosphoramidite approach because it is well established and effective for solid phase synthesis. Previously we studied the effect of chiral amines derived from proline in the phosphoramidite component.[8] Due to the structural properties of the methylphosphonates the tetrazole catalyst is the remaining possibility to influence the coupling reaction. Here we present the use of 5-[2-(2-methoxy)bornyl]tetrazole **1**[12] to



i: 1) $HC(OCH_3)_3$, MeOH, *p*-TsOH, reflux, 2) Na, reflux; ii: $BF_3 \bullet OEt_2$, diethylether, TMSCN; iii: 1) *n*-Bu_3SnN_3, xylene, reflux, or *n*-Bu_3SnCl, NaN_3, xylene, reflux, 2) HCl

SCHEME 1

achieve diastereoselectivity during the coupling reaction starting with a diastereomeric mixture of the commercially available methylphosphoramidites.

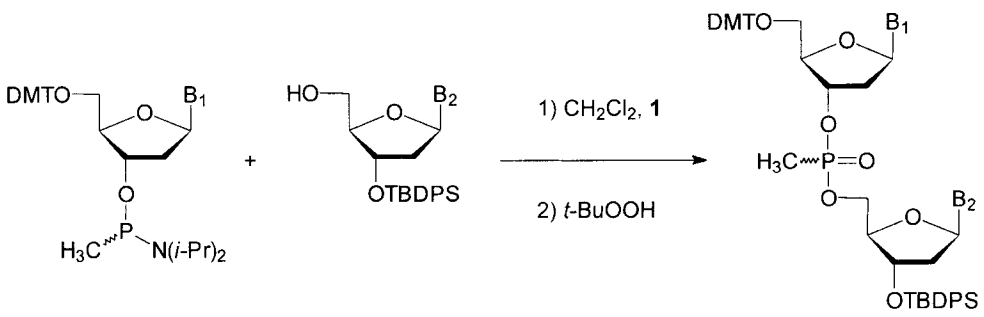
RESULTS AND DISCUSSION

Both enantiomers and the racemic mixture of **1** were used in the coupling reaction. The synthesis of **1** is summarized in scheme 1.

The synthesis starts from camphor **2** which was reacted with trimethylorthoformate in methanol to yield dimethylketal **3**.[13] Unreacted **2** has to be removed by treatment with sodium prior to distillation. **3** was treated with borontrifluoride etherate and trimethylsilylcyanide in diethylether[14] to yield **4** as a mixture of two diastereomers in a 8 : 1 ratio. **4** was converted to title compound **1** in boiling xylene by tri-*n*-butyltinazide which was prepared independently or generated in situ.[15] Treatment with HCl liberated **1**.

Then **1** was used as catalyst for the synthesis of 2′-deoxy dinucleoside methylphosphonates as outlined in scheme 2. The results of the coupling reactions are summarized in table 1.

The coupling reactions with **1** as catalyst were compared with the corresponding reactions catalyzed by 1*H*-tetrazole. Interestingly, not in all cases an effect of **1** on the diastereomeric composition was observed. The reaction seems to be dependent on the



B_1; B_2 = T, A^{Bzl}, G^{ibu} TBDPS = *tert*-butyldiphenylsilyl, DMT = dimethoxytrityl

SCHEME 2

TABLE 1

Catalyst	1*H*-tetrazole	1a	1b	1c
Dimer	**Rp** (ppm)[#] / **Sp** (ppm)	**Rp** (ppm) / **Sp**(ppm)	**Rp / Sp**	**Rp / Sp**
T-T	**46** (31,97) / **54** (32,80)	**45** (31,97) / **55** (32,80)	**46 / 54**	**44 / 56**
T-A	**49** (32,30) / **51** (32,71)	**40** (32,43) / **60** (32,81)	**39 / 61**	**37 / 63**
A-T	**53** (32,11) / **47** (32,89)	**54** (31,95) / **46** (32,70)	**52 / 48**	**51 / 49**
A-A	**59** (32,37) / **41** (32,69)	**48** (32,34) / **52** (32,64)		**42 / 58**
G-T	**50** (32,17) / **50** (32,54)	**64** (32,22) / **36** (32,53)	**63 / 37**	**52 / 48**
G-A *	**46** (32,48) / **54** (32,58)	**35** (32,60) / **65** (32,72)	**38 / 62**	**41 / 59**

[#] ^{31}P chemical shift values ($CDCl_3$) of the corresponding signals. The crude reaction mixtures were measured. Therefore chemical shift values may vary slightly. * In this case the fast isomer (tlc) (normally R*p*) showed the higher chemical shift value. Therefore the assignment of the two diastereomers may be reversed.

nature of the nucleosides. The chirality of the catalyst plays less a role, because there are minor differences in the diastereoselectivity comparing the syntheses of one dimer catalyzed by the tetrazoles **1a-c**.

In conclusion, chiral tetrazoles are interesting candidates for the diastereoselective synthesis of methylphosphonates. Studies on the mechanism of the tetrazole catalyzed coupling reaction and further improvement of the catalyst are in progress.

General procedure for the coupling reaction: 0,03 mmole methylphosphoramidite, 0,03 mmole 3′-O-TBDPS protected nucleoside and 0,12 mmole **1** were dried together in vacuo over P_2O_5 for at least 48 h. The reaction was started by addition of 600 µl of anhydrous CH_2Cl_2 to this mixture. After 2 h the reaction was stopped

by addition of 50 µl of *t*-BuOOH. After 10 min. at room temperature the mixture was diluted with CH_2Cl_2 and extracted with a 1:1 mixture of 5 % aqueous $NaHCO_3$ and 5 % aqueous Na_2SO_3. The aqueous layer was extracted twice with CH_2Cl_2 and the organic layer was dried over Na_2SO_4. After evaporation to dryness the mixture was dissolved in $CDCl_3$ and measured by ^{31}P spectroscopy.

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