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# Synthesis Using Ring Closure Metathesis and Effect on Nucleoside Transport of a (N)-Methanocarba *S*-(4-Nitrobenzyl)thioinosine Derivative

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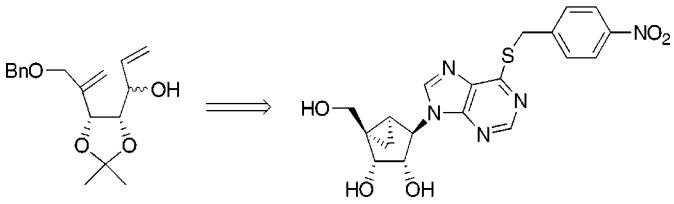
## ABSTRACT




**A new synthetic route to ring-constrained (N)-methanocarba nucleosides and nucleotides is presented. Ring closure of a diene intermediate using Grubbs catalyst provides a new avenue for the preparation of the cyclopentenone derivative 6, which is a versatile intermediate for various carbocycles. The product was almost as potent an inhibitor of *es*-mediated nucleoside transport as the parent compound, inhibiting initial rates of uptake of uridine into cultured CCRF-CEM cells by 50% at approximately 30–50 nM.**

Modulation of adenosine receptors (ARs) and nucleotide (P2) receptors by adenosine derivatives acting as selective agonists and antagonists has the potential for the treatment of wide range of diseases, including those of the cardiovascular, inflammatory, and central nervous systems.[1] A methanocarba approach to conformationally constrain the sugar ring in nucleosides and nucleotides was introduced by Marquez and co-workers.[2] Using this approach, a pseudosugar moiety, based on a bicyclo[3.1.0]hexane template, was fixed in either a Northern ((N), 2′-exo) or Southern ((S), 2′-endo) conformation, according to the pseudorotational cycle. For studies of the ribose ring conformation of nucleosides and nucleotides binding to G protein-coupled receptors (GPCRs), we designed methanocarba analogues. Novel ligands for both ARs[3] and P2[4] receptors, in which the pseudoribose moiety adopted a (N)-conformation, provided favorable receptor affinity and/or selectivity compared to their corresponding (S)-conformers. In binding assays at $A_1$, $A_{2A}$, and $A_3$ARs, (N)-methanocarba-adenosine had a higher affinity than the (S)-analogue, particularly at the human $A_3$AR, with a (N)/(S)-affinity ratio of 150. (N)-Methanocarba analogues con-

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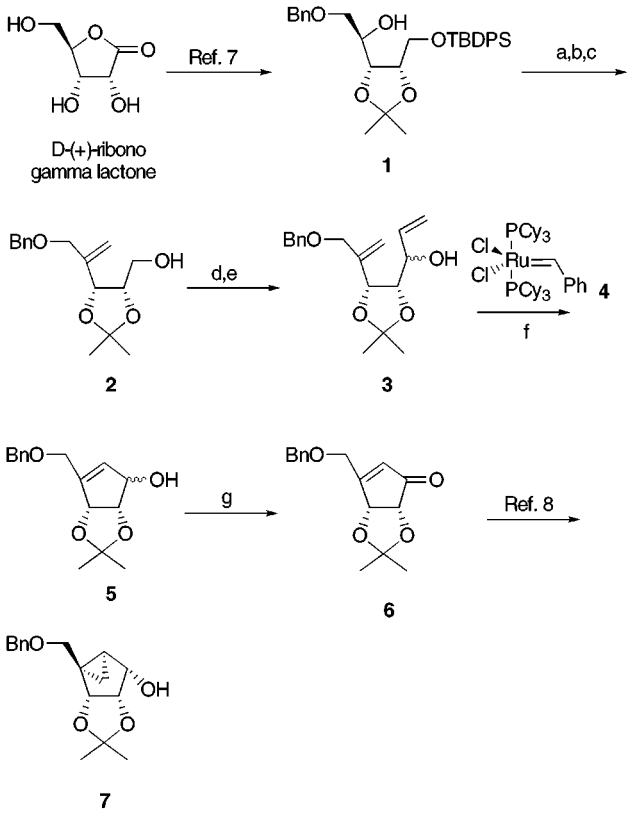
taining various $N^6$-substituents, in which the parent compounds were potent agonists at either $A_1$ (e.g., cyclopentyl) or $A_3$ARs (e.g., 3-iodobenzyl), were found to retain the potency and selectivity in radioligand binding assays.[3] Thus, at least three ARs favored the ribose (N)-conformation.

In this paper we report the efficient synthesis of a (N)-methanocarba *S*-(4-nitrobenzyl)thioinosine (NBTI) derivative[5] as a potential inhibitor of the adenosine uptake carrier, employing a straightforward synthetic strategy. Our approach to the preparation of the key intermediate, **6** (Scheme 1), is

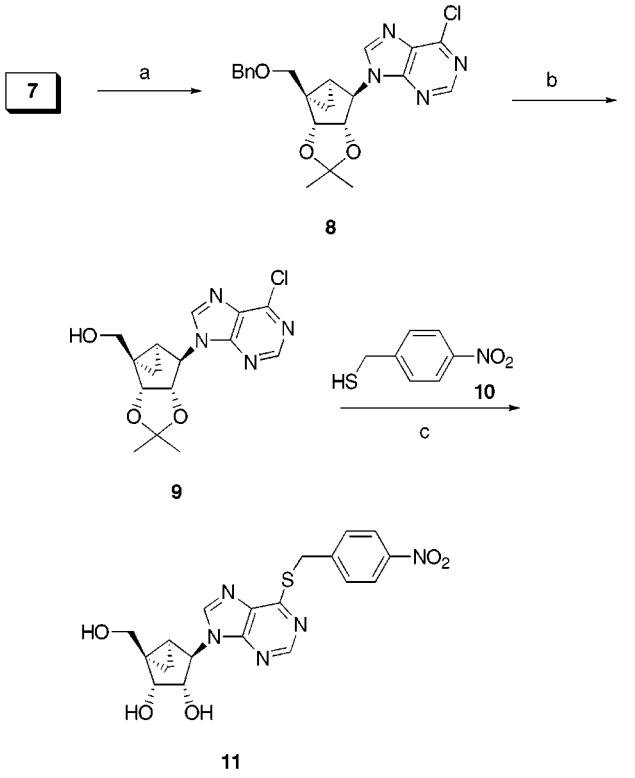
**Scheme 1**[a]




[a] (a) $(COCl)_2$, DMSO, THF, −78 °C, then TEA, rt. (b) $PPh_3CH_3Br$, *n*-BuLi, THF. (c) TBAF, $CH_3CN$. (d) $(COCl)_2$, DMSO, THF, −78 °C, then TEA, rt. (e) Vinylmagnesium bromide, THF, −78 °C. (f) **4**, $CH_2Cl_2$. (g) $MnO_2$, $CHCl_3$.

centered on a ring closing metathesis (RCM) reaction[6] to provide rapid entry into functionalized and enantiomerically pure carbocycles. Starting from the appropriately protected alcohol **1**, which is readily accessible from D-(+)-ribono $\gamma$-lactone in four steps,[7] the required diene, **3**, was constructed in good yield. After oxidation of the *tert*-butyldiphenylsilyl (TBDPS)-protected alcohol, **1**, in Swern conditions, a Wittig reaction was followed by the removal of the TBDPS group using *n*-tetrabutylammonium fluoride to provide the olefinic alcohol, **2**. The necessary precursor, i.e., diene **3**, was then prepared as a diastereomeric mixture by Swern oxidation followed by treatment with 1 M vinylmagnesium bromide in THF at −78 °C. The critical olefin metathesis reaction was accomplished in 80−90% yield by exposure of a dichloromethane solution of diene **3** to 0.2 equiv of the Grubbs catalyst, **4**,[6] for 2 h to give a diastereomeric mixture of cyclopentenols, **5**. The ratio of the alcohols **3** was determined at compound **5** after ring closure reaction (7.3:1, 1*R*-isomer as major). Allylic oxidation of the alcohol **5** using activated $MnO_2$ furnished the corresponding ketone, **6**, in 80% yield. The physical and spectroscopic properties of the synthesized compound, **6**, including optical rotation ($[\alpha]^{20}_D = -7.2$, $c = 1.08$, $CHCl_3$), matched with those reported.[11b] To prepare the ring-constrained bicyclic compound **7**, compound **6** was treated with $NaBH_4$, along with $CeCl_3$, followed by Simmon−Smith cyclopropanation, according to the reported procedure.[8]

**Scheme 2**[a]




[a] (a) 6-Cl-purine, DEAD, $Ph_3P$, THF. (b) (i) $BCl_3$, $CH_2Cl_2$, −78 °C, (ii) DMP, acetone, pTsOH. (c) (i) **10**, NaOMe, MeOH, reflux, (ii) TFA, wet MeOH, 60 °C.

As shown in Scheme 2, the ribose-like moiety **7** was coupled to 6-chloropurine using Mitsunobu conditions to give the fully protected nucleoside **8**,[8] which was then converted to **9** by the treatment with 1 M $BCl_3$ in $CH_2Cl_2$ followed by reforming the acetonide at the 1,2-diol. The target compound,

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**11**,[9] was obtained from **9** by reflux with 4-nitrobenzylthiol, **10**,[10] and sodium methoxide followed by cleavage of the isopropylidene group by trifluoroacetic acid in wet methanol at 60 °C.

Previous syntheses of (N)-methanocarba nucleosides that rely on compound **6** as a starting material have suffered from the lengthy and low-yielding routes to this key intermediate.[11,12] In particular, the route of Johnson and co-workers[11a] to intermediate **6** required a cyclopentenone

intermediate obtained in low yield and featured a stannyl intermediate for introducing the 5′-carbon. The route of Marquez and co-workers[11b] was dependent on a low yield cyclization of a diketophosphonate intermediate, which was complicated by racemization. By our method, it was possible to obtain 1 g of **6** starting from 5 g of D-(+)-ribono $\gamma$-lactone (10.8% overall yield).

The newly synthesized (N)-methanocarba-NBTI derivative, **11**, was examined for its ability to inhibit nucleoside transport by the equilibrative transporters (*es*, *ei*)[13] by measuring initial rates of uptake of 10 $\mu$M $^3$H-uridine in the presence or absence of graded concentrations of either **11** or NBTI (*S*-(4-nitrobenzyl)thioinosine)[5] by cultured human cells that possess only *es* (CCRF-CEM)[14,15] or both *es* and *ei* (HeLa)[16] transporters. The $IC_{50}$ values (concentrations that inhibited transport rates by 50%) calculated from concentration-effect relationships for inhibition of *es*-mediated uridine transport in CCRF-CEM cells by **11** and NBTI were, respectively, 30−50 and 1−5 nM. Compound **11**, like NBTI, had no effect on *ei*-mediated transport of uridine by HeLa cells at concentrations $\leq$1.0 $\mu$M.

Thus, the introduction of a conformationally constrained ribose equivalent satisfied the conformational requirements of the transport-inhibitory site. Compound **11** bound with high affinity, only slightly less than that of NBTI, thereby demonstrating that it, like NBTI, is a useful probe for analysis of the *es* transporter. Further work will be required to determine if other properties of the (N)-methanocarba analogue give it advantages over NBTI as a potential modulator of transport. Compound **11** also bound to the human $A_3AR$[3] with a $K_i$ of 1.97 $\pm$ 0.10 $\mu$M.

In summary, we developed an efficient synthetic methodology for the cyclopentenone derivative **6**, utilizing RCM reaction as a key step, leading to a variety of carbocyclic compounds. During the writing of this paper, Al-Abed and co-workers[17] reported the synthesis of carba-D-arabinose using a similar strategy, including olefin metathesis, starting from a carbohydrate source. In that study, the more reactive and air-sensitive Schrock's catalyst was used.

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**Supporting Information Available:** Experimental procedures and spectroscopic data for compounds **2**−**3**, **5**, **6**, **8**, **9**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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