Nucleosides and Nucleotides. 177. 9-(6,7-Dideoxy-β-D-*allo*-hept-5-ynofuranosyl)adenine: A Selective and Potent Ligand for P_3 Purinoceptor-like Protein[1]

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Adenine nucleosides and nucleotides induce a large variety of physiological responses in various tissues and cells via specific receptors, i.e., purinoceptors, on the cell surface membranes.[2,3] Purinoceptors have been classified into types P_1 and P_2 based on their pharmacological properties. P_1 purinoceptors, which are usually called adenosine receptors, are selective to adenosine and its analogues, while P_2 purinoceptors are specific to adenine nucleotides such as ATP and their analogues. P_1 purinoceptors are subclassified into A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors. P_2 purinoceptors are also subclassified. Although the presence of such purinoceptor subtypes explains most of the functions of purines, it may be necessary to identify additional subtypes to fully explain the functional roles of adenosine and adenine nucleotides in various tissues or cells.

The non-P_1 and non-P_2 muscle relaxant effect of ATP in rabbit thoracic aorta has recently been attributed to a P_3 purinoceptor which is activated by either adenosine or ATP.[4,5] Saitoh and Nakata purified a new [^{3}H]-5′-*N*-ethylcarboxamidoadenosine (NECA) binding protein from rat brain membranes.[6] The ligand specificity of this protein was demonstrated to follow the order NECA > adenosine > inosine > ATP > N^6-cyclopentyladenosine > 2-chloroadenosine, and most xanthines were inactive. This result is very similar to the ranking of potency toward the muscle receptor reported by Chinellato et al. (NECA > adenosine > ATP),[4,5] but is slightly different from the relative order of potency in adrenergic nerves of the rat caudal artery (2-chloroadenosine > ATP > adenosine).[7] Therefore, this [^{3}H]NECA binding protein from the rat brain membranes was thought to be a subtype of P_3 purinoceptor or P_3 purinoceptor-like protein (P_3LP). Since the physiological roles of P_3LP and P_3 purinoceptor have not been fully elucidated, we need a specific ligand to obtain further information regarding this receptor. In this communication, we describe the structural requirements of various 5′-modified adenosine analogues with regard to P_3LP binding and present a new specific ligand, 9-(6,7-dideoxy-β-D-*allo*-hept-5-ynofuranosyl)adenine (**17a**).

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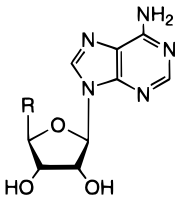
Table 1. P_3 Purinoceptor-like Protein (P_3LP) Binding Activity of Various 5′-Modified Adenosine Analogues[a]

compds	K_i (nM)	compd	K_i (nM)
1	37 ± 1.2	**9**	1100 ± 520
2	51 ± 12	**10**	280 ± 38
3	64 ± 20	**11**	64 ± 12
4	21000 ± 7300	**17a**	19 ± 8.9
5	84 ± 4.1	**17b**	450 ± 140
6	81 ± 2.5	**18a**	100 ± 57
7	54 ± 4.5	**18b**	5600 ± 1700
8	70 ± 25		

[a] P_3LP binding activity to [^{3}H]NECA (40 nM) was determined as described previously.[6] P_3LP was partially purified by hydroxylapatite chromatography from a CHAPS-solubilized preparation of rat brain membranes. A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors and adenotin were not present in this preparation. K_i values were expressed as mean ± SEM (n = 3).



	R		R
1	CONHEt	7	CH_2SPh
2	CONHMe	8	CH_2SCH_2Ph
3	$CONH_2$	9	CH_2S^nPr
4	$CONMe_2$	10	C≡CH
5	CH_2Cl	11	C≡N
6	CH_2N_3		

Figure 1. Structures of compounds **1**−**11**.

P_3LP binding assays were carried out using P_3LP that was partially purified from rat brain membranes by [^{3}H]-NECA (40 nM) in the presence of various concentrations of adenosine analogues.[6] K_i values were calculated using Graph Pad software (ISI Software), and the results are summarized in Table 1. Since P_3LP binds tightly to NECA, we examined several NECA analogues with regard to P_3LP binding. NECA (**1**), 5′-*N*-methylcarboxamidoadenosine (**2**), and 5′-carboxamidoadenosine (**3**) were found to be good ligands, with K_i values of 37, 51, and 64 nM, respectively. Compounds with a longer alkyl group at the carboxamide nitrogen showed slightly better activity. However, 5′-*N*,*N*-dimethylcarboxamidoadenosine (**4**) did not bind well (K_i = 21 μM). Therefore, the terminal bulky alkyl group at the carboxamide nitrogen is believed to be well-tolerated and the NH group may play an important role in binding to P_3LP. To obtain further information regarding the binding region around the 5′-position of adenosine, we tested other 5′-deoxy-5′-substituted analogues of adenosine which did not have an acidic proton. Compounds **5**, **6**, **7**, **8**, and **11** (Figure 1) also showed potent inhibitory activities, with K_i values in the nanomolar range. However, compounds **9** and **10** were less effective inhibitors than the other compounds. Therefore, some bulky substituents can be well accommodated at the 5′-position in P_3LP. To further clarify whether an acidic proton of the substituent plays an important role and since, among the compounds tested thus far, NECA binds most potently to P_3LP, we designed **17a** and its diastereomer **17b** and their congeners **18a**,**b**.

Chemistry

The synthesis of the target compounds was straightforward, as shown in Scheme 1. Treatment of N^6-

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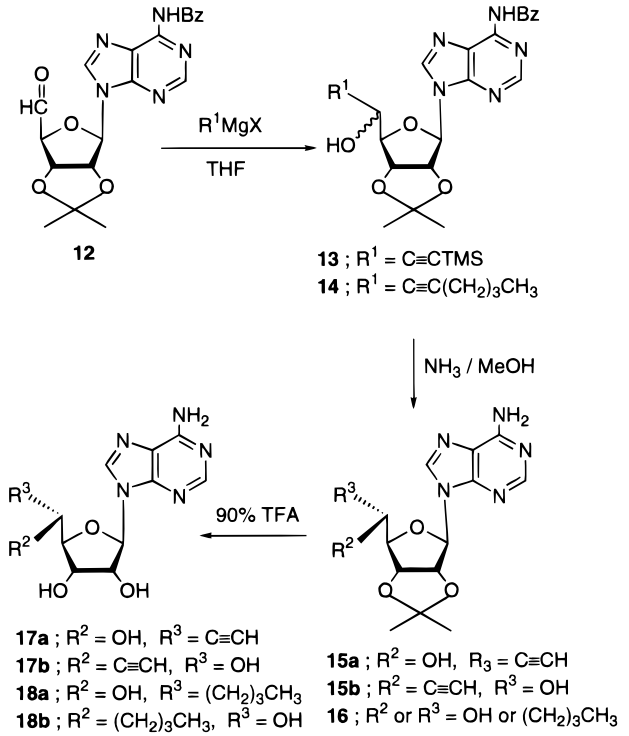
Table 2. Affinities of Ligands to Adenosine A_1, A_{2A}, A_{2B}, and A_3 Receptors and P_3 Purinoceptor-like Protein (P_3LP)[a]

	K_i (nM)				
compd	P_3LP[b]	A_1[c]	A_{2a}[c]	A_{2b}[c]	A_3[d]
17a	19 ± 8.9	33% inhibn at 10 μM	40% inhibn at 100 μM	no inhibn at 100 μM	no inhibn at 10 μM
17b	450 ± 140	21% inhibn at 10 μM	40% inhibn at 100 μM	no inhibn at 100 μM	no inhibn at 10 μM
NECA	37 ± 1.2	6.3[e]	10[e]	2600[e]	110[f]

[a] K_i values were expressed as mean ± SEM ($n = 3$). [b] See Table 1 for the binding assay. [c] The activities of A_1 and A_{2A} adenosine receptors were measured by binding [^{3}H]DPCPX (2 nM) and [^{3}H]CGS 21680 (5.9 nM) to rat brain membranes, respectively. A_{2B} adenosine receptor activity was determined by measuring cAMP production in VA-13 cells.[10] [d] The activity of A_3 adenosine receptor was measured by binding [^{125}I]AB-MECA (0.15 nM) to RBL-2H3 cell membranes in the presence of 100 nM DPCPX and 100 μM AppNHp (A) and in the presence of 100 μM DPCPX, 100 μM AppNHp, and 10 nM IB-MECA (B). Specific A_3 adenosine receptor activity was calculated from the difference between the binding activity in (A) and (B). [e] Data taken from ref 10. [f] Data taken from ref 12.

Scheme 1



benzoyl-2′,3′-*O*-isopropylideneadenosine-5′-aldehyde (**12**) with TMSC≡CMgBr (3 equiv, prepared from TMSC≡CH and EtMgBr) at −20 °C in THF gave a diastereomixture of **13** (*R*:*S* = about 2:1, determined by its ^{1}H NMR) in 70% yield, which was partially separable by silica gel column chromatography. However, the stereochemistry at the 5′-position of **13** could not be determined at this stage. A mixture of less polar **13** with a amall amount of the more polar one was treated with NH_3 in MeOH (saturated at 0 °C) for 26 h at room temperature to give crystalline **15a** in 74% yield. X-ray structural analysis of **15a**[8] showed that **15a** has an *R* configuration at the 5′-position. Compound **15a** was further treated with aqueous 90% trifluoroacetic acid to give **17a**[9] in 79% yield. 9-(6,7-Dideoxy-α-L-*talo*-hept-5-ynofuranosyl)adenine (**17b**) was also obtained by the same sequential deprotection of more polar **13**. To further study the structure−activity relationship, 9-(7-*C*-butyl-6,7-dideoxy-*β*-D-*allo*-hept-5-ynofuranosyl)adenine (**18a**) and its diastereomer **18b** were synthesized in a similar way starting from **12**, as shown in Scheme 1.

Biological Evaluation and Discussion

The most potent compound in the series of 5′-modified adenosine analogues in P3 purinoceptor-like protein binding was found to be **17a**, with a K_i value of 19 nM, while its 5′ diastereomer **17b** was 24-fold less effective (Table 1). A similar tendency for stereoselective recognition at the 5′-position by P_3LP was also found in **18a**,**b** although **18a** and **18b** were 5-fold and 63-fold less effective than **17a**, respectively. Therefore, P_3LP recognizes not only the stereochemistry of the hydroxyl group at the 5′-position but also the bulkiness of the substituents. Again, an acidic proton of the substituent plays an important role.

We next compared the receptor selectivity for selected nucleosides **17a**,**b** and NECA against P_1 purinoceptor subtypes, such as adenosine A_1, A_{2A}, A_{2B}, and A_3 receptors, since **17a**,**b** are just adenosine analogues. As shown in Table 2, **17a** showed almost no inhibitory activity against A_{2A}, A_{2B}, and A_3 receptors, although it inhibited only slightly [^{3}H]DPCPX binding against adenosine A_1 receptor (33% inhibition at 10 μM). Compound **17b** was also a selective ligand for P_3LP, but much less potent for P_3LP binding than **17a**. NECA showed a high affinity to P_3LP, but was a nonselective adenosine receptor agonist. Therefore, **17a** is the first selective and potent ligand for P_3LP.

In summary, we have identified the first selective and potent ligand for P_3 purinoceptor-like protein. Further studies will be needed to elucidate the structure−activity relationships of adenosine analogues against P_3-LP in greater detail, particularly with regard to the pharmacological activity of **17a**.

Supporting Information Available: X-ray crystallographic data of **15a** and the synthetic methods described Scheme 1 (10 pages). Ordering information is given on any current masthead page.

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(8) $C_{15}H_{17}N_5O_4$, $M = 331.33$, orthorhombic, $P2_12_12_1$, $a = 10.068(2)$ Å, $b = 21.536(4)$ Å, $c = 7.444(1)$ Å, $V = 1613.9$ Å^3, $D_{calcd} = 1.363$ g cm^{-1}. A total of 1367 independent reflections were collected and used for the structure analysis. The final R value was 0.034.

(9) Data: mp 242−243 °C; LRMS (EI) m/z 291 (M^+); ^{1}H NMR (DMSO-d_6 + D_2O) 8.27 (s, 1 H, H-2), 8.11 (s, 1 H, H-8), 5.88 (d, 1 H, H-1′, $J_{1',2'} = 7.3$ Hz), 4.63 (dd, 1 H, H-2′, $J_{2',1'} = 7.3$, $J_{2',3'} = 4.6$ Hz), 4.45 (dd, 1 H, H-5′, $J_{5',4'} = 4.0$, $J_{5',C\equiv CH} = 2.0$ Hz), 4.19 (dd, 1 H, H-3′, $J_{3',2'} = 4.6$, $J_{3',4'} = 1.3$ Hz), 3.96 (dd, 1 H, H-4′, $J_{4',5'} = 4.0$, $J_{4',3'} = 1.3$ Hz), 3.35 (d, 1 H, C≡CH, $J_{C\equiv CH,5'} = 2.0$ Hz). Anal. ($C_{12}H_{13}N_5O_4 \cdot 0.25H_2O$) C, H, N.

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