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Novel nucleotide triphosphates as potent $P2Y_2$ agonists

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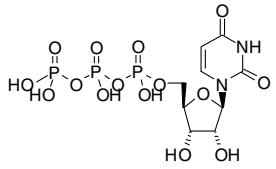
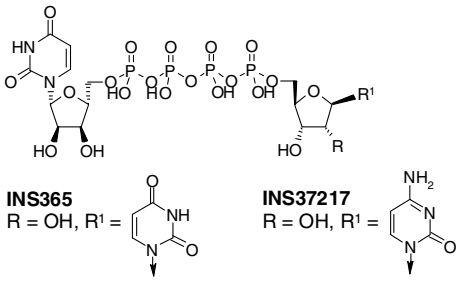
Abstract—The synthesis and $P2Y_2$ activities of a novel series of nucleoside triphosphates are described. Many of these compounds were potent agonists of the $P2Y_2$ receptor.

The P2Y receptors are a group of 7-transmembrane G-protein coupled receptors that are activated by purine and pyrimidine nucleotides. Several human subtypes have been cloned and characterised.[1] Activation of $P2Y_2$ receptors in the airway epithelium increases mucus secretions, cilia beat frequency and transport of chloride ions and water across the luminal surface, leading to an increase in the rate of mucociliary clearance. In the conjunctiva $P2Y_2$ activation stimulates tear secretion from lacrimal tissues. A $P2Y_2$ agonist may therefore provide a useful therapy in diseases such as cystic fibrosis and dry eye.[2]



UTP

UTP is a potent natural agonist for the $P2Y_2$ receptor, but is chemically unstable and readily metabolised and therefore has a very short duration of action. Various dinucleotide polyphosphates have been investigated as purinergic agonists with improved stability over UTP.[3] The $P2Y_2$ agonists INS365 (diquafosol tetrasodium) and INS37217 (denufosol tetrasodium) are in development for dry eye disease and cystic fibrosis, respectively.[4]



Our objective was to identify some novel $P2Y_2$ agonists with potencies similar to that of UTP. We therefore prepared some UTP analogues in which the triphosphate and ribose units were retained, but incorporating some 'unnatural' bicyclic aromatic bases in place of the uracil.

A group of available 6,6 and 6,5 fused bicyclic pyridones was reacted with bis(trimethylsilyl)acetamide in acetonitrile, followed by tribenzoyl protected ribofuranose acetate and tin tetrachloride. The products of this reaction were treated with sodium methoxide in methanol to remove the benzoyl esters, and the resulting nucleosides converted to triphosphates using standard conditions.[5] The desired triphosphates were separated from the crude reaction mixtures by preparative HPLC and isolated as the ammonium salts (Scheme 1). Incorporation of the triphosphate unit was confirmed by LC-MS and ^{31}P NMR spectroscopy. The ^{31}P NMR pattern characteristic of a triphosphate (doublet, doublet, triplet, $J = 20$ Hz) could clearly be seen in each case (Fig. 1). The $P2Y_2$ agonist potencies of the resulting triphosphates are shown in Table 1.

Keywords: $P2Y_2$; SAR; Potent agonists; Stability.
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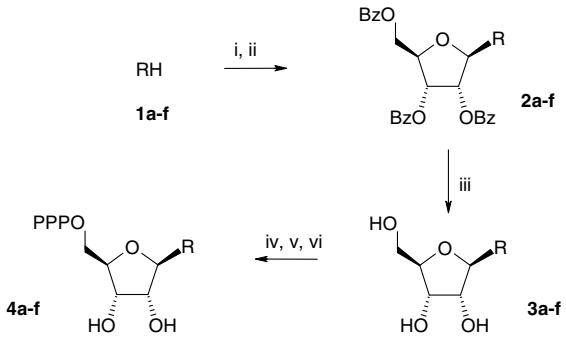


Scheme 1. Reagents: (i) BSA, MeCN, (ii) β-*O*-ribofuranose-1-acetate-2,3,5-tribenzoate, $SnCl_4$; (iii) NaOMe, MeOH; (iv) $POCl_3$, $P(OMe)_3$, proton sponge; (v) $H_4P_2O_7{\cdot}1.5(Bu_3N)$, Bu_3N, DMF; (vi) NH_4Cl, H_2O.

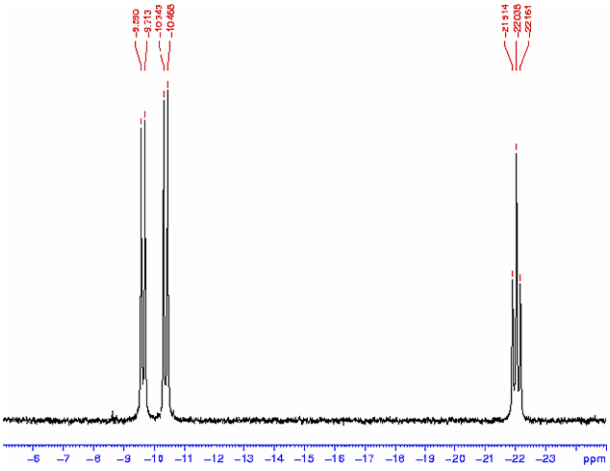
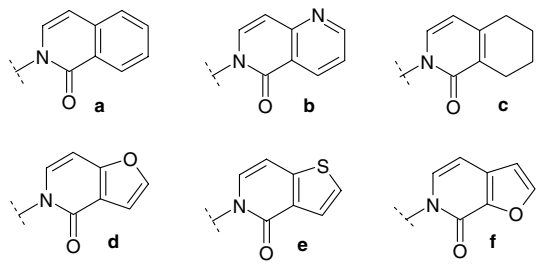


Figure 1. ^{31}P NMR spectrum of a triphosphate.

Table 1. Agonist potencies of compounds **4a–4f** compared with UTP and INS365



Compound	$P2Y_2$ EC_{50}[6] (nM)
4a	31
4b	35
4c	55
UTP	7
4d	68
4e	30
4f	221
INS365	100[4a]

The isocarbostyril **4a**, pyridyl pyridone **4b** and thienyl pyridone **4e** were all sub 50 nM agonists of $P2Y_2$. The partially reduced compound **4c** and the furanyl pyridone **4d** were only slightly less potent, while **4f**, the regioisomer of **4d**, was significantly less active.

We next investigated the positional effect of a methyl group around the isocarbostyril template. 4-Methyl isocarbostyril was prepared by the reaction of allyl amine with 2-iodobenzoyl chloride followed by a palladium-catalysed cyclisation, while 5-, 6-, 7- and 8-methyl isocarbostyrils were prepared[7] from cinnamic acids via a Curtius rearrangement or by rearrangement of isoquinoline-*N*-oxides (Scheme 2). These were converted to the nucleotide triphosphates **5a**–**5e** following the procedure in Scheme 1.

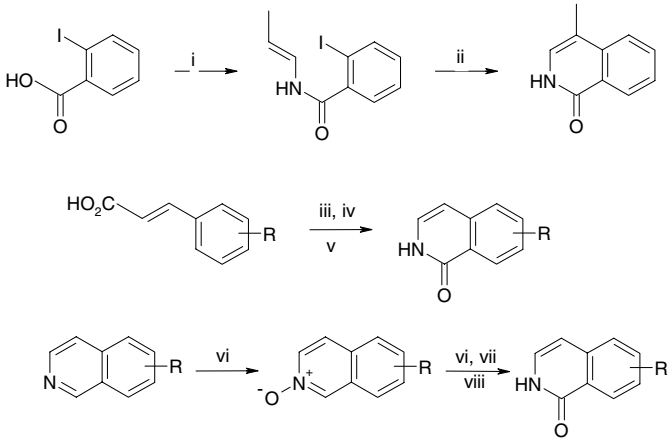
The $P2Y_2$ agonist potencies of these compounds are shown in Table 2. Methylation at the 4-position was very detrimental to activity. The 6-, 7- and 8-positions were tolerant of substitution, though in no case was potency improved compared with the unsubstituted compound **4a**.

To explore the SAR around the 6- and 7-positions, some isocarbostyrils with a variety of 6- and 7- substitutents were prepared[7] from cinnamic acids or isoquinoline-*N*-oxides, and converted to the nucleotide triphosphates **5f**–**5p** as described in Scheme 1. The $P2Y_2$ agonist potencies of these compounds are shown in Table 3. The only examples not to be prepared directly from the appropriate substituted isocarbostyrils were the cyano compounds, prepared by treating the appropriate tribenzoyl-protected bromo-substituted isocarbostryil nucleosides with copper cyanide in DMF, and the sulfone, by oxidation of the thioether. Some disubstituted compounds were prepared from the 6,7-difluoro isocarbostyril nucleoside by nucleophilic displacement of the 6-fluorine (Scheme 3).

Although the 6-methyl compound **5c** was significantly more active than the 7-methyl compound **5d**, it was found that substitution with fluoro, chloro and cyano in the 6-position (**5f**, **5g**, and **5h**) gave less active compounds than the corresponding 7-isomers (**5i**, **5j**, and **5k**). These offered excellent agonist potencies, comparable to that of UTP. The 7-methoxy compound **5l** gave no advantage over the unsubstituted isocarbostyril **4a**, and substituting at the 7-position with a thioether or a sulfone (**5m** and **5n**) reduced potency. However, 6-,7-disubstitution with a thioether, dimethyl amino or methoxy group at the 6-position and fluorine at the 7-position (**5q**, **5r**, and **5s**) again provided compounds with potencies similar to that of UTP; these were some of the most active $P2Y_2$ agonists synthesised in our laboratory.

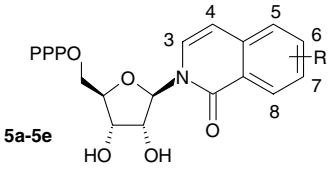
Some key compounds were tested against three other purinergic receptor subtypes, $P2Y_1$, $P2Y_4$ and $P2Y_6$. The results of these experiments are shown in Table 4.

Whilst UTP has agonist activity at $P2Y_4$ and $P2Y_6$ receptors, and INS365 at $P2Y_4$, our isocarbostyril-substituted



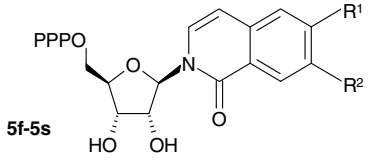
Scheme 2. Reagents and conditions: (i) allyl amine, Et_3N, DCM; (ii) $Pd(PPh_3)_4$, Et_3N, Bu_4NCl, DMF; (iii) Et_3N, ethyl chloroformate, acetone, 0 °C; (iv) NaN_3, H_2O; (v) $(C_6H_5)_2CH_2$, Bu_3N, toluene, 190 °C; (vi) *m*-CPBA, DCM; (vii) Ac_2O, reflux; (viii) NaOH, H_2O.

Table 2. Agonist potencies of compounds **5a**–**5e**

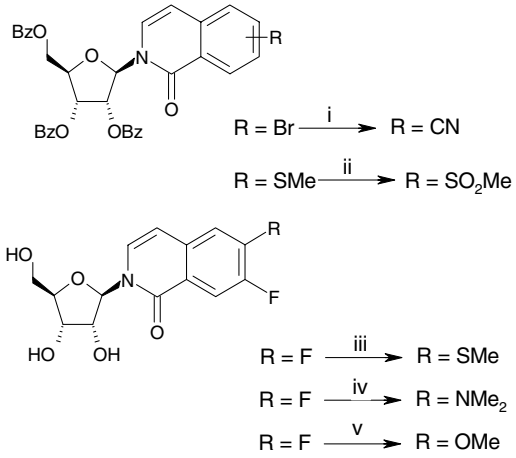


Compound	R	$P2Y_2$ EC_{50}[6] (nM)
5a	4-Me	17,500
5b	5-Me	557
5c	6-Me	35
5d	7-Me	148
5e	8-Me	85

Table 3. Agonist potencies of 6- and 7-substituted isocarbostyrils **5f**–**5s**



Compound	R^1	R^2	$P2Y_2$ EC_{50}[6] (nM)
5f	F	H	77
5g	Cl	H	541
5h	CN	H	88
5i	H	F	5
5j	H	Cl	10
5k	H	CN	12
5l	H	OMe	26
5m	H	SMe	140
5n	H	SO_2Me	650
5o	Cl	Cl	13
5p	F	F	16
5q	SMe	F	3
5r	NMe_2	F	4
5s	OMe	F	6



Scheme 3. Reagents and condition: (i) CuCN, DMF, reflux; (ii) *m*-CPBA, DCM; (iii) NaSMe, EtOH, reflux; (iv) Me_2NH, H_2O, 90 °C; (v) NaOMe, MeOH, reflux.

Table 4. Agonist potencies against some other P2Y receptor subtypes

Compound	$P2Y_1$ EC_{50}[8] (nM)	$P2Y_4$ EC_{50}[8] (nM)	$P2Y_6$ EC_{50}[8] (nM)
UTP	>2000	39	424
INS365	>20,000	400[4a]	>20,000[4a]
4a	>20,000	7043	>20,000
5i	>20,000	3583	>20,000
5j	>20,000	>20,000	>20,000
5p	>20,000	>20,000	>20,000
5q	>20,000	>20,000	>20,000

nucleotide triphosphates had little or no activity against these isoforms and thus provide a more selective template than the uracil-derived $P2Y_2$ agonists. All compounds tested were inactive against $P2Y_1$.

In conclusion, a selection of novel UTP analogues bearing 'unnatural' bicyclic bases in place of the uracil have been synthesised and their $P2Y_2$ agonist potencies investigated. Several of these compounds had activity comparable to that of UTP, and some key examples showed

excellent selectivity for $P2Y_2$ over $P2Y_4$ and $P2Y_6$. Our isocarbostyril nucleotide triphosphate template thus provided some novel, potent and selective $P2Y_2$ agonists.

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