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A General Approach toward the Synthesis of *C*-Nucleoside Pyrazolo[1,5-*a*]-1,3,5-triazines and Their 3′,5′-Bisphosphate C-Nucleotide Analogues as the First Reported in Vivo Stable $P2Y_1$-Receptor Antagonists

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In our effort to identify potent purinergic $P2Y_1$ receptor antagonists as potent platelet aggregation inhibitors with enhanced metabolic stability, we developed an efficient route for the large-scale preparation of 2′-deoxy-*C*-nucleosides of pyrazolo[1,5-*a*]-1,3,5-triazine. The key strategic elements of this novel synthetic approach involved the following: (i) the use of a novel activating group, the *N*-methyl-*N*-phenylamino group, which was easily generated in high yield by treatment of the pyrazolo[1,5-*a*]-1,3,5-triazin-4-one (**5**) with phosphorus oxychloride and dimethylaniline under high pressure, (ii) a regio- and stereospecific palladium-mediated coupling reaction of the readily available unprotected glycal 1,4-anhydro-2-deoxy-D-*erythro*-pent-1-enitol (**4b**) and the 8-iodo derivative (**16**), and (iii) the stereoselective reduction of the ketone group of the furanosyl ring followed by the subsequent displacement of the *N*-methyl-*N*-phenylamino group upon treatment with methylamine. The β configuration at the anomeric C-1′ position of the glycal moieties was perfectly retained throughout this conversion. This procedure afforded 8-(2′-deoxy-β-D-ribofuranosyl)-2-methyl-4-(*N*-methylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (**21**) and 8-(2′-deoxy-β-D-xylofuranosyl)-2-methyl-4-(*N*-methylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (**24**) with an overall yield of 50% and 39%, respectively. Finally, the conversion of nucleosides **21** and **24** to the pyrazolotriazine *C*-nucleotides 3′,5′-bisphosphate **2** and 3′,5′-cyclophosphate **26** is also described herein and represents the first reported nucleotide derivatives within the pyrazolo[1,5-*a*]-1,3,5-triazine series. Preliminary biological testing has shown that compound **2** strongly inhibits ADP-induced human platelet aggregation and shape change and possesses significant efficacies 30 min after injection in rat, highlighting a strong $P2Y_1$-receptor antagonist activity in vitro combined with a prolonged duration of action in vivo.

Introduction

In our research program studying the synthesis and biological evaluation of novel 2′-deoxyadenosine-3′,5′-bisphosphates as $P2Y_1$-receptor antagonists, we recently reported the synthesis of 2,N^6-dimethyl-2′-deoxyadenosine-3′,5′-bisphosphate (**1b**, Figure 1), a potent and selective $P2Y_1$-receptor antagonist.[1] Preliminary in vivo testing has shown that this compound possesses a very brief activity as an anti-aggregating agent. Thus, the synthesis of the corresponding isosteric compound **2** (Figure 1) possessing significantly better in vivo activities represented a very attractive challenge.[2−8] In recent years a number of modified 2′-deoxynucleoside analogues based on structural modifications of the sugar have been described.[9−11] However, until now, no proof of better in

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(1) Raboisson, P.; Baurand, A.; Cazenave, J.-P.; Gachet, C.; Retat, M.; Spiess, B.; Bourguignon, J.-J. *J. Med. Chem.* **2002**, *45*, 962−972.

(2) Ravi, R. G.; Kim, H. S.; Servos, J.; Zimmermann, H.; Lee, K.; Maddileti, S.; Boyer, J. L.; Harden, T. K.; Jacobson, K. A. *J. Med. Chem.* **2002**, *45*, 2090−2100.

(3) Gachet, C. *Thromb. Haemost.* **2001**, *86*, 222−232.

(4) Hechler, B.; Leon, C.; Vial, C.; Vigne, P.; Frelin, C.; Cazenave, J. P.; Gachet, C. *Blood* **1998**, *92*, 152−159.

(5) Léon, C.; Hechler, B.; Freund, M.; Eckly, A.; Vial, C.; Ohlmann, P.; Dierich, A.; LeMeur, M.; Cazenave, J.-P.; Gachet, C. *J. Clin. Invest.* **1994**, *104*, 1731−1737.

(6) Fabre, J.-E.; Nguyen, M.; Latour, A.; Keifer, J. A.; Audoly, L. P.; Coffman, T. M.; Koller B. H. *Nat. Med.* **1999**, *5*, 1199−1202.

(7) Léon, C.; Freund, M.; Ravanat, C.; Baurand, A.; Cazenave, J.-P.; Gachet, C. *Circulation* **2001**, *103*, 718−723.

(8) Baurand, A.; Raboisson, P.; Freund, M.; Léon, C.; Cazenave, J. P.; Bourguignon, J. J.; Gachet, C. *Eur. J. Pharmacol.* **2001**, *412*, 213−221.

(9) Nandanan, E.; Jang, S.-Y.; Moro, S.; Kim, H. O.; Siddiqui, M. A.; Russ, P.; Marquez, V. E.; Busson, R.; Herdewijn, P.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. *J. Med. Chem.* **2000**, *43*, 829−842.

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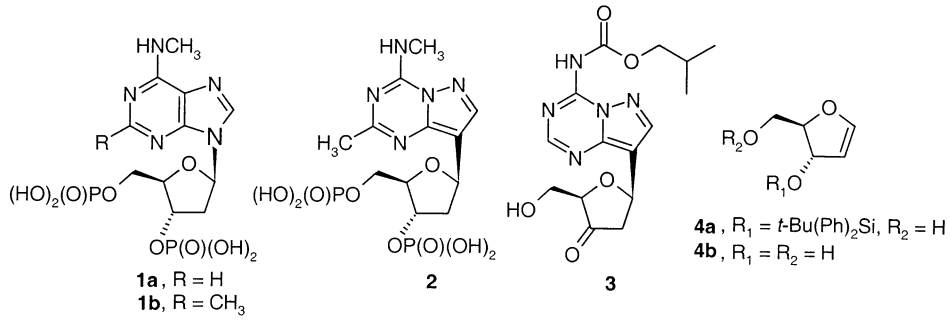


FIGURE 1.

vivo activities was reported. Thus, *C*-nucleotide analogues have become a focus of intense investigation with the rationale that replacing the glycosidic carbon−nitrogen linkage by the carbon−carbon bond would increase the metabolic stability of these derivatives toward nucleosidase enzymes that cleave the link between the base and the sugar moiety.[12] To investigate the scope of such an approach, we have now extended our investigation of the pyrazolo[1,5-*a*]-1,3,5-triazine-3′,5′-bisphosphate *C*-nucleotides and we report herein the details of the synthetic studies which have successfully led to the large-scale preparation of the 8-(2′-deoxy-*β*-D-ribofuranosyl)-2-methyl-4-(*N*-methylamino)pyrazolo[1,5-*a*]-1,3,5-triazine-3′,5′-bisphosphate (**2**). Subsequently, the $P2Y_1$ receptor antagonist activities were also evaluated in vitro and in vivo.

Two main synthetic approaches leading to pyrazolo-[1,5-*a*]-1,3,5-triazine *C*-nucleosides were described in the literature.[13−17] The first started from a substituted pyrazole nucleus followed by triazine ring construction.[13−15] More recently, Zhang and co-workers reported an interesting two-step synthesis of the 8-(2′-deoxy-*β*-D-*glycero*-pentofuran-3′-ulos-1′-yl)-4-(*N*-isobutyloxycarbonyl)aminopyrazolo[1,5-*a*]-1,3,5-triazine **3** (Figure 1), which is based upon a regio- and stereospecific Pd-mediated cross-coupling reaction (PMCCR) of a 3-substituted *tert*-butyldiphenylsilyl glycal **4a**[16] (Figure 1) with a protected 8-iodo-N^4-diisobutyloxycarbonylamino derivative.[17] It was well-accepted that in this PMCCR of the furanoid glycal **4**, the substituted hydroxyl groups exert a directing effect on the attack of the organopalladium reagent on the enol ether double bond and, thereby, determine the stereochemistry of the resulting *C*-nucleoside product.[18,19] When a monoprotected glycal was used, PMCCR occurred from the opposite face of the glycal ring that is substituted, yielding a single *C*-nucleosidic product.[17−19] However, although the unique case of the PMCCR described in the literature with the unprotected furanoide **4b**[21] and mercuric acetate derivative had led to a poor *α*/*β*-*C*-glycosides selectivity (a 3/4 ratio was encountered),[18] no other example with different aglycon and/or different reaction conditions was previously reported.

Nevertheless, neither of these routes are readily amenable to synthesis of N^4-substituted derivatives because the exclusively accessible 4-substituent obtained after blocking and unblocking strategy would be the NH_2 group.[17] Additional steps would be necessary to introduce other substituents in this position, resulting in a long synthetic route, loss in yields, and a potential scrambling of the anomeric center of the carbohydrate moiety.[20] Moreover, the preparation of the ribosyl starting material **4a**[16] is not conveniently accessible, and was obtained through a 4-step synthesis and required a final deprotection step at the end of the reaction pathway.[16,17] These disadvantages limited the scope of this procedure in *C*-nucleoside synthesis, in particular for their use in parallel synthesis and multigram scale reactions.

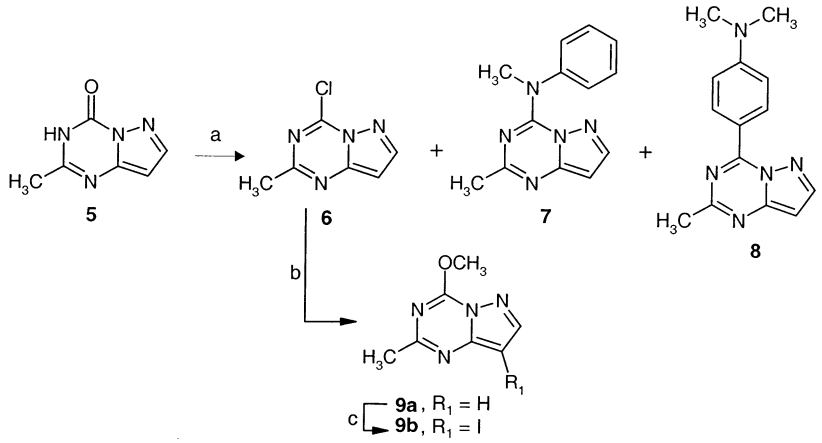
With the aim of expanding the usefulness of this approach, we decided to explore the possibility of introducing an activating group at position 4 that is stable under the PMCCR and which could be displaced by various amines at the final step of the synthesis. To avoid the use of the hardly accessible glycal **4a**,[16] we sought to use the unsubstituted furanoside 1,4-anhydro-2-deoxy-D-*erythro*-pent-1-enitol **4b**,[21] which is readily prepared in 2 steps from the cheap and commercially available thymidine,[22] as the starting material in the synthesis of *C*-nucleoside pyrazolo[1,5-*a*]-1,3,5-triazine derivatives.

Results and Discussion

1. Optimized 4-Activating Group. The 8-iodo-4-methoxy-2-methylpyrazolo[1,5-*a*]-1,3,5-triazine (**9b**, Scheme 1) represented an interesting candidate for the exploration of the coupling reaction with the unsubstituted furanoid **4b**,[21] because of the potential to readily displace

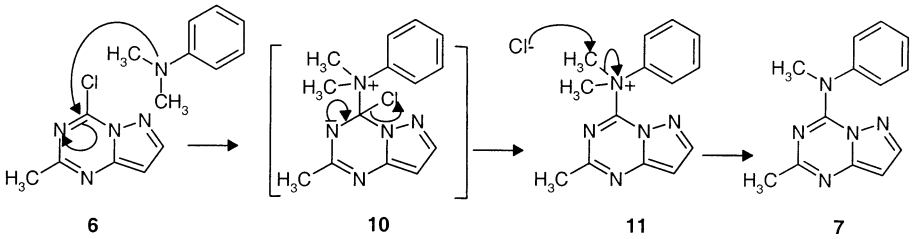
(10) Kim, Y.-C.; Gallo-Rodriguez, C.; Jang, S.-Y.; Nandanan, E.; Adams, M.; Harden T. K.; Boyer, J. L.; Jacobson, K. A. *J. Med. Chem.* **2000**, *43*, 746−755.
(11) Kim, H. S.; Ravi, R. G.; Marquez, V. E.; Maddileti, S.; Wihlborg, A.-K.; Erlinge, D.; Malmsjö, M.; Boyer, J. L.; Harden, T. K.; Jacobson, K. A. *J. Med. Chem.* **2002**, *45*, 208−218.
(12) Abusamhadneh, E.; McDonald, N. E.; Kline P. C. *Plant Sci.* **2000**, *153*, 25−32.
(13) Tam, S. Y.-K.; Hwang, J. S.; De Las Heras, F. G.; Klein, R. S.; Fox, J. J. *J. Heterocycl. Chem.* **1976**, *13*, 1305−1308.
(14) Chu, C. K.; Watanabe, K. A.; Fox, J. J. *J. Heterocycl. Chem.* **1980**, *17*, 1435−1439.
(15) Tam, S. Y.-K.; Klein, R. S.; Wempen, I.; Fox, J. J. *J. Org. Chem.* **1979**, *44*, 4547−4553.
(16) Farr, R. N.; Daves, G. D., Jr. *J. Carbohydr. Res.* **1990**, *9*, 653−660.
(17) Zhang, H.-C.; Brakta, M.; Daves, G. D., Jr. *Nucleosides Nucleotides* **1995**, *14*, 105−116.
(18) Hacksell, U.; Daves, G. D., Jr. *J. Org. Chem.* **1983**, *48*, 2870−2876.
(19) Cheng, J. C.-Y.; Hacksell, U.; Daves, G. D., Jr. *J. Org. Chem.* **1986**, *51*, 3093−3098.
(20) Zhang, H.-C.; Daves, G. D., Jr. *J. Org. Chem.* **1992**, *57*, 4690−4696.
(21) Cheng, J. C.-Y.; Hacksell, U.; Daves, G. D., Jr. *J. Org. Chem.* **1985**, *50*, 2778−2780.
(22) Cameron, M. A.; Cush, S. B.; Hammer, R. P. *J. Org. Chem.* **1997**, *62*, 9065−9069.

SCHEME 1. Activation of 2-Methylpyrazolo[1,5-*a*]-1,3,5-triazin-4-one 5 with *N*,*N*-Dimethylaniline in Refluxing Phosphorus Oxychloride[a]



[a] Key: (a) $POCl_3$, *N*,*N*-dimethylaniline, 87%; (b) MeOH, 54%; (c) NIS, 68%.

SCHEME 2



the methoxy group at the end of the reaction sequence. Attempts to drive this reaction using a previously described experimental procedure[17] were not successful and led to a complex mixture of unseparable and unidentified products. However, LC-MS analysis showed a signal at 374, highlighting the susceptibility of the methoxy compound **9b** (Scheme 1) to a displacement by the free hydroxyl groups of glycal **4b** (Figure 1). Therefore, it was of great interest to develop a new and efficient activating group for the nucleophilic replacement of *C*-nucleoside that would offer the necessary stability under the PMCCR of those previously reported.

The reaction of 2-methylpyrazolo[1,5-*a*]-1,3,5-triazin-4-one (**5**, Scheme 1)[23] with *N*,*N*-dimethylaniline in refluxing phosphorus oxychloride afforded the desired 4-chloro derivative **6** as the major product in 87% yield, together with traces of the two byproducts **7** and **8** which were obtained in small amounts (<5%, Scheme 1). These two unexpected products were purified and analyzed and their structures were unambiguously assigned by elemental analysis and mass and NMR spectroscopy. The ^{1}H NMR spectra of the first byproduct exhibited an NCH_3 signal at δ 3.76 ppm and a phenyl group at δ 7.17−7.44 ppm. The mass spectrum and the microanalysis data were in perfect accordance with the structure of **7**. Moreover, the structure of compound **7** was identical in all respects with the product prepared through the reaction of iminochloride **6** and *N*-methylaniline.

We postulate that the reaction leading to **7** occurs through a thermal promoted nucleophilic addition/elimination sequence of the dimethylaniline as depicted in Scheme 2. The first step likely involves formation of a dimethylphenylammonium intermediate (**10**, Scheme 2), generated by the *N*-attack of the dimethylaniline followed by a demethylation and a subsequent rearomatization of the triazine ring, leading to the thermodynamically more stable *N*-methyl-*N*-phenylamino adduct **7**. A distant analogy to the second step of the proposed mechanism is the reported heteroatom-demethylation with trimethylsilyl chloride.[24] Similar *N*-methyl-*N*-phenylamino adducts have been previously observed in the literature.[25,26]

The other side product obtained during the preparation of the iminochloride **6** exhibited an $N(CH_3)_2$ signal at δ 3.16 ppm and an AB system at δ 7.87 ppm ($\Delta\delta$ = 1.37, J_{AB} = 9.5 Hz). The mass as well as the microanalysis data supported the structure of the 4-(*N*,*N*-dimethylamino)-phenyl derivative **8** (Schemes 1 and 3). This product is not stable under strong aqueous acidic conditions and led to two identified hydrolysis products: the 3-aminopyrazole **14** and the *N*-acetyl-4-(*N*,*N*-dimethylamino)benzamide (**15**, Scheme 3). We suggest that this addition of dimethylaniline under the iminochloride **6** leading to a C−C bond formation proceeds as shown in Scheme 3, through an aromatic nucleophilic substitution. Interestingly, this example is the first reported C−C coupling reaction between a halogenated aromatic ring and dimethylaniline.

To support the two proposed mechanisms depicted in Schemes 2 and 3 involving a nucleophilic attack of the

(23) Robins, R. K.; Revankar, G. R.; O'Brien, D. E.; Springer, R. H.; Novinson, T.; Albert, A.; Senga, K.; Miller, J. P.; Streeter, D. G. *J. Heterocycl. Chem.* **1985**, *22*, 601−634.

(24) Olah, G. A.; Narang, S. C.; Balaram Gupta, B. G.; Malhorta, R. *J. Org. Chem.* **1979**, *44*, 1247−1251.

(25) Robins, R. K. *J. Am. Chem. Soc.* **1956**, *78*, 784−790.

(26) Hull, R. *J. Chem. Soc.* **1959**, 481−484.

SCHEME 3

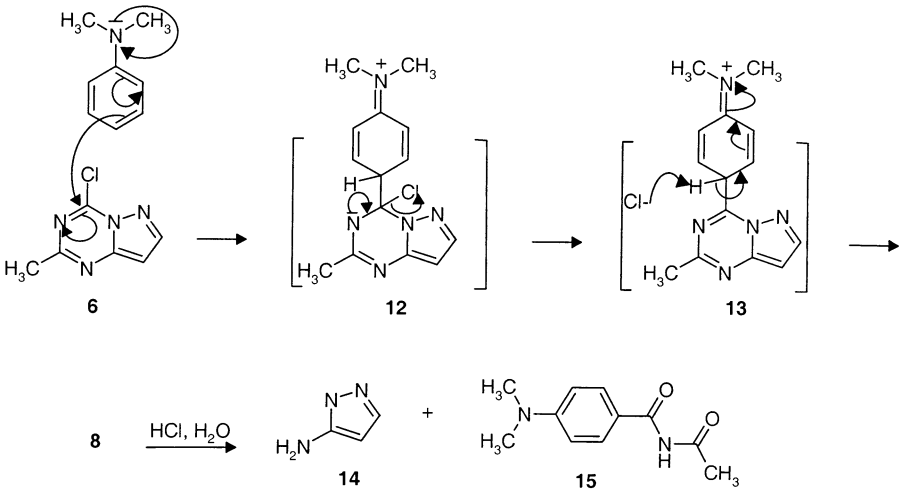


TABLE 1. Synthesis of Compounds 7 and 8 under Different Experimental Conditions

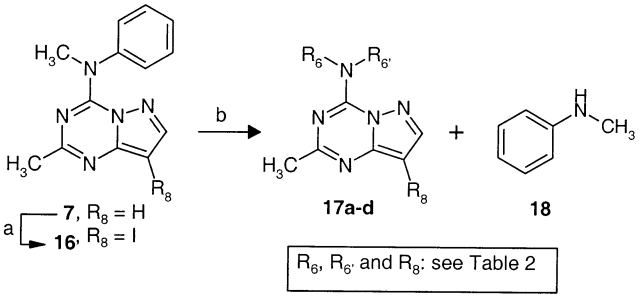
	yield (%)[b]	
conditions[a]	**7**	**8**
a	<5	<5
b	10	7
c	35	21
d	92	<5

[a] Conditions: (a) **5**, $POCl_3$, *N*,*N*-dimethylaniline, reflux, 4 h; (b) **6**, *N*,*N*-dimethylaniline, $CHCl_3$, reflux, 24 h; (c) **5**, $POCl_3$, *N*,*N*-dimethylaniline, sealed tube, 125 °C, 4 h; (d) (1) **5**, $POCl_3$, *N*,*N*-dimethylaniline, reflux, 2 h, (2) *N*-methylaniline, triethylamine, CH_2Cl_2, 0 °C to room temperature. [b] Yields of isolated products.

dimethylaniline on the 4-chloro derivative **6**, a pure sample of **6** was heated under refluxing chloroform with freshly distilled dimethylaniline. Under these conditions, the chloro derivative **6** slowly disappears and undergoes a coupling reaction with the excess of dimethylaniline present leading to byproducts **7** and **8** (conditions b, Table 1).

However, since dimethylaniline is a very weak nucleophile, an increase in the temperature and pressure of the reaction was necessary to accelerate the rate of the reaction. Thus, as shown in Table 1, when 2-methylpyrazolo[1,5-*a*]-1,3,5-triazin-4-one (**5**)[23] was treated with an excess of *N*,*N*-dimethylaniline and phosphorus oxychloride in a sealed tube at 125 °C over 4 h (conditions c), the starting material was completely consumed and the two stable target dimethylaniline adducts **7** and **8** were obtained respectively in 35% and 21% yields. Finally, the *N*-methyl-*N*-phenylamino derivative was obtained in excellent yield (92%) when the quite unstable iminochloride **6** was directly treated in situ without purification with *N*-methylaniline at 0 °C (conditions d). Moreover, the pK_a of compound **7** (close to 3.0) and its high stability in the presence of water or refluxing ethanol make the *N*-methyl-*N*-phenylamino group a suitable candidate for nucleophilic addition−elimination displacements. To our satisfaction, treatment of **7** with methylamine as a typical nucleophile amine led to the desired *N*-methylamino derivative **17a** (Scheme 4, Table 2) in 78% isolated yield. Although this sequence required extended reaction times at room temperature presenting a drawback for an expeditious preparation of 4-substited derivatives (after 2 days, only 10% of the desired product was obtained), the reaction times could be shortened to 1 h by running the reaction in a sealed tube. As shown in Table 2, the facile displacement of the *N*-methyl-*N*-phenylamino group with various amines should be useful as it opens up access to a wide range of N-4 substituted compounds.

SCHEME 4. Nucleophilic Displacement of the *N*-Methyl-*N*-phenylamino Group by Various Amines[a]



[a] Key: (a) NIS, 87%; (b) $HNR_6R_{6'}$, 53−98%.

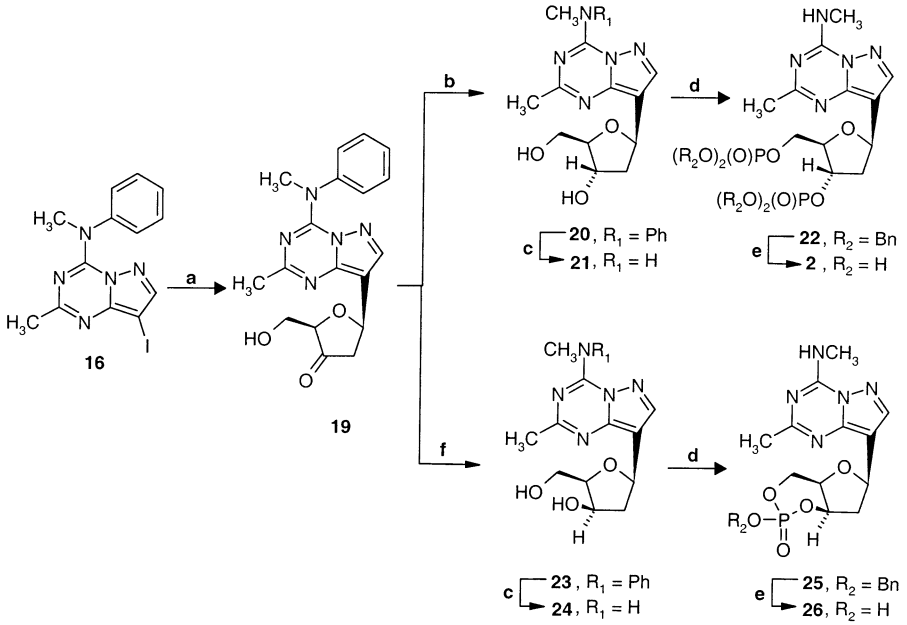
TABLE 2. Displacement of the 4-(*N*-Methyl-*N*-phenylamino) Group with Various Amines[a]

compd	R_6	$R_{6'}$	R_8	yield (%)[b]
17a	CH_3	H	H	78
17b	H	H	I	53
17c	Bn	H	I	98
17d	$-(CH_2)_4-$		I	92

[a] Conditions: $R_6R_{6'}NH$, EtOH, 100 °C, sealed tube, 1 h. [b] Yields of isolated products.

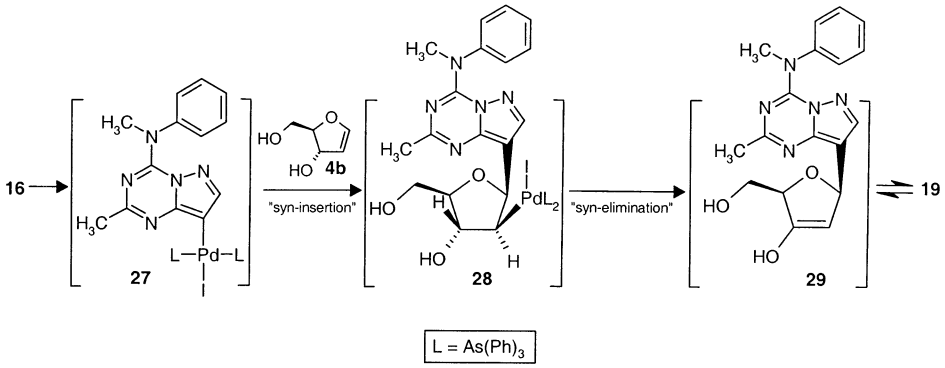
2. Application to *C*-Nucleoside Preparation. Having demonstrated the viability of this approach employing the *N*-methyl-*N*-phenylamino group as an activating group with derivatives **17a**−**d** (Table 2), its application to the preparation of *C*-nucleosides was also assessed. Initial studies demonstrated that the direction of attack by the organopalladium reagent on the furanoid enol ether double bond is highly dependent on the relative steric bulks of the C3 and C4 substituents of glycals **4**.[18,19] Unexpectedly, the palladium-mediated coupling of the 8-iodo derivative **16** and the readily available unprotected glycal 1,4-anhydro-2-deoxy-D-*erythro*-pent-1-enitol (**4b**),[21]

SCHEME 5[a]



[a] Key: (a) **4b**, bis(dibenzylideneacetone)Pd(0), Ph_3As, TEA, 75%; (b) sodium triacetoxyborohydride, 92%; (c) NH_2CH_3, 67−73%; (d) *t*-BuOK, TBPP, 63−66%; (e) H_2, Pd/C, 91−92%; (f) K-selectride, 78%.

SCHEME 6. Proposed Mechanism for the PMCCR



using the conditions depicted in the literature,[17] led exclusively to an adduct in 75% isolated yield that could be assigned as the *β*-*C*-nucleoside **19** (Scheme 5). The coupling reaction was accompanied by aglycon deiodination leading to **7** (15%). Convincing evidence for the *β*-anomeric configuration assigned to nucleoside **19** was obtained from the comparison of their ^{1}H NMR coupling constants $J_{1'2'a}$, $J_{1'2'b}$ (6.6 and 10.6 Hz, respectively), which are very close to those of the known *β*- nucleosides.[17] Moreover, the ^{1}H spectrum exhibited signals for the anomeric hydrogen (H-1′) at *δ* 5.39 ppm assigned to the *β* anomer.[20] To the best of our knowledge, this is the only instance in which a product resulting from organopalladium attack on a single face of unsubstituted glycal **4b** has been isolated. However, the conditions used in the present study were different from those reported in the literature, employing glycal **4b**, mercuric acetate salts, and palladium II.[19] Thus, we postulated that the more bulky Palladium ligands (triphenylarsine), which were used in the present study, make the organopalladium reagent **27** (Scheme 6), which was obtained via an initial transmetalation, a very hindered complex that is probably not able to attack the olefinic glycal **4b** through the face bearing the 3-hydroxyl group. Therefore, in the opposite of the example reported in the literature using less bulky ligands,[19] the PMCCR selectively proceeds via a syn-addition on the sterically most open face of **4b** (the face α) leading to adduct **28**, which subsequently undergoes a syn-elimination of the hydridopalladium (Scheme 6). Finally, a tautomerism equilibrum would afford the single target compound **19**.

Stereospecific reductions of the 3′-keto group of intermediate **19** with sodium triacetoxyborohydride[16,27] yielded the 2′-deoxyribofuranosyl *C*-nucleoside **20** in 92% yield while K-selectride permitted reduction by the less sterically hindered face of the glycal ring[16] leading to **23** (78% isolated yield). Finally, the *N*-methyl-*N*-phenylamino group was easily displaced with methylamine, with retention of stereochemistry of the 2′-deoxyfuranosyl moiety. Thus, an efficient preparation of the *C*-nucleoside analogues **21** and **24** (Scheme 5) was accomplished.

3. *C*-Nucleotide Preparation. Phosphorylation of **21** with tetrabenzylpyrophosphate (TBPP),[28,29] followed by

(27) Gribble, G. W.; Ferguson, D. C. *J. Chem. Soc., Chem. Commun.* **1975**, 535−536.

catalytic hydrogenolysis, afforded the expected 2-methyl-*C*-nucleotide derivative **2** in 91% yield (Scheme 5). Attempts to apply this same methodology to the preparation of the xylofuranosyl derivative failed and the cyclic compound **25** was obtained as the major product, highlighting the proximity of the two hydroxy groups in the cis configuration which facilitate the cyclization. As such, hydrogenolysis of compound **25** led to the corresponding cyclic phosphate derivative **26** in 92% yield.

4. Biological Evaluation. The new derivatives **2** (Figure 1) and **26** (Scheme 5) prepared in the present study were first tested as antagonists in a platelet aggregation assay as described earlier.[1] Even though **2** exhibited strong antiaggregating properties (pA2 = 6.5 ± 0.3) identical with those of the $P2Y_1$ receptor reference antagonist MRS-2179 ($pA_2 = 6.55 \pm 0.05$),[29] the cyclic derivative **26** was found to be inactive. As seen with compounds **1** (Figure 1) no agonist activity was observed.[1] Moreover, the intracellular Ca^{2+} rise induced in washed human platelets by 5 μM ADP could be totally inhibited by 100 μM **2**, in the presence or absence of 2 mM external Ca^{2+}. Finally, studies in rat showed that ADP-induced platelet aggregation was still inhibited 30 min after injection (38% inhibition of aggregation to ADP 1 μM), revealing that the in vitro potency of compound **2** was sustained in vivo.

Conclusions

In summary, we have disclosed herein a very efficient and particularly attractive route toward the large-scale preparation of 2′-deoxy-*C*-nucleosides of the pyrazolo[1,5-*a*]-1,3,5-triazine and their *C*-nucleotide derivatives. The impetus for this work was the easy access to the 4-(*N*-methyl-*N*-phenylamino)pyrazolo[1,5-*a*]-1,3,5-triazine derivatives (e.g. **7**), obtained by treatment of the corresponding pyrazolo[1,5-*a*]-1,3,5-triazin-4-one (**5**) with phosphorus oxychloride and dimethylaniline, and our observation of the facile nucleophilic displacement of this optimized *N*-methyl-*N*-phenylamino activating group with amines. Another main advantage of this approach is the use of the readily available 1,4-anhydro-2-deoxy-D-*erythro*-pent-1-enitol (**4b**)[21] as a source of glycal in a regio- and stereoselective palladium-mediated cross-coupling reaction with the 8-iodo-2-methyl-4-(*N*-methyl-*N*-phenylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (**16**) permitting a multigram scale synthesis of *C*-furanosyl-4-one compounds **19**. Moreover, displacement of the *N*-methyl-*N*-phenylamino group at the end of the reaction represents a more concise and general route for the introduction of substitutions and functionalities at the 4-position, thus avoiding additional protection/deprotection steps which can compromise the integrity of the anomeric center. The conversion of so-formed *C*-nucleoside 8-(2′-deoxy-β-D-ribofuranosyl)-2-methyl-4-(*N*-methylamino)-pyrazolo[1,5-*a*]-1,3,5-triazine (**21**) and 8-(2′-deoxy-β-D-xylofuranosyl)-2-methyl-4-(*N*-methylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (**24**) to corresponding *C*-nucleotide 3′,5′-bisphosphate **2** and 3′,5′-cyclophosphate **26** with use of the tetrabenzylpyrophosphate procedure was also demonstrated. Preliminary biological evaluations have also revealed that compound **2** inhibits ADP-induced platelet aggregation (pA2 = 6.5 ± 0.3) and shape change in vitro and possesses significant efficacies 30 min after injection in rat. Thus, compound **2** appears to be the most stable and one of the more potent $P2Y_1$ receptor antagonists known to date and represents a very promising pharmacological tool to obtain a better understanding of physiology and physiopathology involving ADP-promoted aggregation through the $P2Y_1$ receptor pathway.

Experimental Section

Chemical Synthesis. With the exception of THF and Et_2O, all solvents were obtained from commercial suppliers and used without further purification. These two solvents were freshly distilled from sodium benzophenone ketyl. Flash chromatography was performed on Si 60 (40−63 μm) silica gel. Thin-layer chromatography was carried out using Silica gel 60 F_{254} plates. The spots were visualized either under UV light (λ = 254 nm) or by spraying with molybdate reagent (H_2O/concentrated H_2SO_4/$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$/$(NH_4)_2\cdot Ce(SO_4)_4\cdot 2H_2O$, 90/10/25/1, v/v/w/w) and charring at 140 °C for a few minutes. All chemical yields are unoptimized and generally represent the result of a single experiment. ^1H NMR were recorded on a 200- or 300-MHz spectrophotometer at room temperature. Chemical shifts are given in ppm (δ), coupling constants (*J*) are in hertz (Hz), and signals are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; quint, quintuplet; m, multiplet; br s, broad singlet. Melting points are uncorrected. Elemental analyses were performed by the CNRS department of microanalysis (CNRS, Vernaison, France) and are indicated only by the elemental symbols within ±0.4% of the theoretical values unless otherwise noted.

2-Methyl-4-(*N*-methyl-*N*-phenylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (7) and 2-Methyl-4-[4-(*N*,*N*-dimethylamino)phenyl]pyrazolo[1,5-*a*]-1,3,5-triazine (8). Method A. A solution of **5**[12] (1.0 g, 6.66 mmol), *N*,*N*-dimethylaniline (3.0 mL, 23.7 mmol), and phosphorus oxychloride (8 mL, 107 mmol) was stirred at 125 °C in a sealed tube for 4 h. After the solution was cooled to room temperature, the excess of phosphorus oxychloride was removed under vacuum. The mixture was taken up with ice-cooled water (100 mL) and the resulting solution was neutralized with saturated $NaHCO_3$, then extracted with ethyl acetate (3 × 100 mL). The organic layer was dried (Na_2SO_4) and concentrated to dryness under reduced pressure. Chromatography on silica (EtOAc/hexanes, 1:1) afforded the pure compounds **7** (555 mg, 35%) and **8** (355 mg, 21%). Compound **7**: colorless solid; mp 116 °C (Et_2O/hexane). ^1H NMR (300 MHz, $CDCl_3$) δ 2.58 (s, 3H), 3.76 (s, 3H), 6.26 (d, *J* = 1.9, 1H), 7.17−7.44 (m, 5H), 7.71 (d, *J* = 1.9, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.0, 42.5, 94.5, 126.3, 126.4, 127.4, 129.4, 145.2, 149.6, 151.9, 163.0. FABMS *m*/*z* 239 $(M)^+$. Anal. Calcd for $C_{13}H_{13}N_5$: C, 65.26; H, 5.48; N, 29.27. Found: C, 65.35; H, 5.50; N, 29.23. Compound **8**: colorless solid, mp 123 °C (Et_2O/hexane). ^1H NMR (300 MHz, $CDCl_3$) δ 2.75 (s, 3H), 3.16 (s, 6H), 6.52 (d, *J* = 2.2, 1H), 7.87 (AB system, Δδ = 1.37, J_{AB} = 9.5, 4H), 8.20 (d, *J* = 2.2, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.2, 40.4, 95.1, 111.3, 117.0, 133.7, 146.7, 151.5, 153.7, 153.8, 162.3. FABMS *m*/*z* 254 $(M + H)^+$. Anal. Calcd for $C_{14}H_{15}N_5$: C, 66.38; H, 5.97; N, 27.65. Found: C, 66.27; H, 5.99; N, 27.51.

Method B. A solution of **5**[12] (1.0 g, 6.66 mmol), *N*,*N*-dimethylaniline (3.0 mL, 23.7 mmol), and phosphorus oxychloride (8 mL, 107 mmol) was refluxed for 2 h. After the solution was cooled to room temperature, the excess of phosphorus oxychloride was removed under reduced pressure. Then, the residue was redissolved in CH_2Cl_2 (40 mL), and *N*-methylaniline (1.5 mL, 11.8 mmol) was added dropwise with stirring at 0 °C under argon. After 10 min, the reaction mixture was allowed to warm to room temperature. After evaporation of the solvent, the residue was chromatographed on silica

(28) Vacca, J. P.; deSolms, S. J.; Huff, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 3478−3479.
(29) Yu, K.-L.; Fraser-Reid, B. *Tetrahedron Lett.* **1988**, *29*, 979−982.

(EtOAc/hexanes, 1:1) to give **7** (1.46 g, 92%), which was identical in all respects with the compound that was obtained through Method A.

4-Methoxy-2-methylpyrazolo[1,5-*a*]-1,3,5-triazine (9a). A solution of **5**[12] (2.70 g, 18.0 mmol), phosphorus oxychloride (30 mL, 401 mmol), and *N*,*N*-dimethylaniline (8 mL, 63.2 mmol) was refluxed for 1.5 h. The excess of phosphorus oxychloride was then removed under vacuum. The residue was poured onto ice−water (30 mL), then the solution was extracted with EtOAc (3 × 40 mL), dried (Na_2SO_4), and concentrated to dryness under reduced pressure. The residue was directely put into MeOH (40 mL). After 12 h of stirring at 20 °C, the solvent was evaporated to dryness and the residue was dissolved in ice-cold EtOAc (50 mL). This solution was successively washed with cold, saturated $NaHCO_3$ (50 mL), brine (40 mL), and finally water (30 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Recrystallization from Et_2O/hexanes yielded compound **9a** (1.60 g, 54%) as a yellow solid. ^{13}C NMR ($CDCl_3$, 75 MHz) δ 25.8, 56.5, 96.3, 147.2, 151.4, 153.6, 163.5. EIMS *m*/*z* 165 (M + H)$^+$. Anal. Calcd for $C_7H_8N_4O$: C, 51.21; H, 4.91; N, 34.13. Found: C, 51.28; H, 4.95; N, 34.42.

8-Iodo-4-methoxy-2-methylpyrazolo[1,5-*a*]-1,3,5-triazine (9b). A solution of **9a** (300 mg, 1.83 mmol) and *N*-iodosuccinimide (493 mg, 2.19 mmol) in chloroform (20 mL) was stirred under argon for 3 h at reflux. After evaporation of the solvent, the residue was diluted with EtOAc (30 mL), washed with saturated sodium bisulfite (10 mL) and cold water (20 mL), dried (Na_2SO_4), then concentrated to dryness under reduced pressure. Chromatography on silica (EtOAc/hexanes, 1:1) afforded compound **9b** (361 mg, 68%) as a colorless solid. ^1H NMR (200 MHz, $CDCl_3$) δ 2.67 (s, 3H), 4.32 (s, 3H), 8.07 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.1, 49.0, 57.1, 150.3, 151.1, 153.5, 165.2. EIMS *m*/*z* 291 (M + H)$^+$. Anal. Calcd for $C_7H_7IN_4O$: C, 28.99; H, 2.43; N, 19.32. Found: C, 29.03; H, 2.54; N, 19.21.

8-Iodo-2-methyl-4-(*N*-methyl-*N*-phenylamino)pyrazolo-[1,5-*a*]-1,3,5-triazine (16). To a solution of **7** (1.00 g, 4.18 mmol) in dry chloroform (100 mL) was added *N*-iodosuccinimide (1,33 g, 5.91 mmol), and the mixture was refluxed for 0.5 h. After the mixture was cooled at room temperature, the solvent was evaporated in vacuo. Then, the residue was partitioned between dichloromethane (100 mL) and water (70 mL). The aqueous layer was extracted twice with additional dichloromethane (2 × 50 mL). The combined organic phases was washed with 10% sodium bisulfite (70 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude material was purified by column chromatography on silica (EtOAc/CH_2Cl_2/hexanes, 2:3:5). Recrystallization from ethanol yielded compound **16** (1.33 g, 87%) as colorless crystals: mp 192 °C. ^1H NMR (200 MHz, $CDCl_3$) δ 2.66 (s, 3H), 3.76 (s, 3H), 7.16−7.44 (m, 5H), 7.71 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.3, 42.7, 47.4, 126.5, 127.6, 129.4, 144.9, 148.9, 149.5, 164.7. EIMS *m*/*z* 366 (M + H)$^+$. Anal. Calcd for $C_{13}H_{12}IN_5$: C, 42.76; H, 3.31; N, 19.18. Found: C, 42.54; H, 3.38; N, 19.04.

2-Methyl-4-(*N*-methylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (17a). A solution of **7** (300 mg, 1.26 mmol) and methylamine (2 M, 2.0 mL, 4.0 mmol) in ethanol (10 mL) was stirred at 100 °C in a sealed tube for 12 h. After the mixture was cooled the solvent was evaporated under reduced pressure. The crude reaction product was purified by column chromatography on silica gel (EtOAc/CH_2Cl_2/EtOH, 4:5:1). Recrystallization from ethanol and diethyl ether yielded compound **17a** (160 mg, 78%) as colorless crystals: mp: 165 °C. ^1H NMR (200 MHz, $CDCl_3$) δ 5.44 (s, 3H), 5.70 (d, J = 1.9, 3H), 6.89 (d, J = 0.9, 1H), 6.98 (br s, 1H), 7.52 (d, J = 0.9, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.3, 27.6, 95.5, 145.4, 149.5, 149.6, 164.1. EIMS *m*/*z* 164 (M + H)$^+$. Anal. Calcd for $C_7H_9N_5$: C, 51.52; H, 5.56; N, 42.92. Found: C, 51.20; H, 5.61; N, 42.90.

4-Amino-8-iodo-2-methylpyrazolo[1,5-*a*]-1,3,5-triazine (17b). A solution of **16** (350 g, 0.95 mmol) and 28% ammonium hydroxide (2.0 mL, 15.9 mmol) in ethanol (10 mL) was stirred at 110 °C in a sealed tube for 14 h. After the solution was cooled to room temperature, the solvent was evaporated under reduced pressure. The crude reaction product was purified by column chromatography on silica gel (EtOAc/CH_2Cl_2/EtOH, 4:5:1). Recrystallization from ethanol and diethyl ether yielded compound **17b** (138 mg, 53%) as colorless crystals: mp 220 °C. ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 2.36 (s, 3H), 7.00 (br s, 2H), 7.78 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.1, 48.6, 149.5, 150.0, 151.2, 165.2. EIMS *m*/*z* 276 (M + H)$^+$. Anal. Calcd for $C_6H_6IN_5$: C, 26.20; H, 2.20; N, 25.47. Found: C, 25.94; H, 2.21; N, 25.46.

4-(*N*-Benzylamino)-8-iodo-2-methylpyrazolo[1,5-*a*]-1,3,5-triazine (**17c**) was prepared from **16** and benzylamine as described for **17b**: a colorless solid (98%); mp 142 °C. ^1H NMR (300 MHz, $CDCl_3$) 2.62 (s, 3H), 4.85 (d, J = 5.9, 2H), 6.76 (br s, 1H), 7.35−7.42 (m, 5H), 7.92 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6, 45.1, 47.9, 128.3, 128.5, 129.3, 137.0, 148.9, 149.3, 149.4, 165.8. EIMS *m*/*z* 366 (M + H)$^+$. Anal. Calcd for $C_{13}H_{12}IN_5$: C, 42.75; H, 3.31; N, 19.18. Found: C, 42.86; H, 3.36; N, 19.07.

8-Iodo-2-methyl-4-(1-pyrrolidinyl)pyrazolo[1,5-*a*]-1,3,5-triazine (**17d**) was prepared from **16** and pyrrolidine as described for **17b**: colorless solid (92%); mp 127 °C. ^1H NMR (200 MHz, $CDCl_3$) δ 1.95−2.10 (m, 4H), 2.54 (s, 3H), 3.70−4.60 (m, 4H), 7.88 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 46.6, 50.3, 50.8, 148.5, 148.6, 151.5, 165.5. EIMS *m*/*z* 330 (M + H)$^+$. Anal. Calcd for $C_{10}H_{12}IN_5$: C, 36.49; H, 3.67; N, 21.28. Found: C, 36.34; H, 3.64; N, 20.75.

2-Methyl-4-(*N*-methyl-*N*-phenylamino)-8-(*β*-D-*glycero*-pentofuran-3′-ulos-1′-yl)pyrazolo[1,5-*a*]-1,3,5-triazine (19). Bis(dibenzylideneacetone)palladium(0) (310 mg, 0.53 mmol) and triphenylarsine (330 mg, 1.08 mmol) were mixed in acetonitrile (50 mL) and stirred under nitrogen at 25 °C for 15 min. The complex was then transferred by syringe to a solution of 1,4-anhydro-2-deoxy-D-*erythro*-pent-1-enitol (**4b**; 800 mg, 6.89 mmol), 8-iodo-2-methyl-4-(*N*-methyl-*N*-phenylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (**16**; 2.47 g, 6.76 mmol), and tri-*n*-butylamine (1.89 mL, 7.92 mmol) in acetonitrile (150 mL). The reaction mixture was stirred under nitrogen at 60 °C for 18 h. After the solution was cooled to room temperature, the solvent was evaporated under reduced pressure. The crude reaction product was purified by column chromatography on silica gel (EtOAc/hexanes, 1:1). Recrystallization from ethyl acetate and hexanes yielded compound **19** (1.80 g, 75%) as colorless crystals: mp 70 °C. ^1H NMR (200 MHz, $CDCl_3$) δ 2.55 (s, 3H), 2.85 (ddd, J = 6.6, J = 10.6, J = 17.8, 2H), 3.74 (s, 3H), 3.86−4.11 (m, 3H), 5.39 (dd, J = 6.6, J = 10.6, 1H), 6.06 (dd, J = 10.3, J = 2.7, 1H), 7.14−7.45 (m, 2H), 7.31−7.45 (m, 3H), 7.66 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.7, 42.7, 45.1, 63.5, 71.3, 82.7, 107.7, 126.6, 127.8, 129.4, 143.9, 144.8, 148.9, 149.5, 163.6, 214.9. EIMS *m*/*z* 354 (M + H)$^+$. Anal. Calcd for $C_{18}H_{19}N_5O_3$: C, 61.18; H, 5.42; N, 19.82. Found: C, 61.41; H, 5.46; N, 20.11.

8-(2′-Deoxy-*β*-D-ribofuranosyl)-2-methyl-4-(*N*-methyl-*N*-phenylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (20). Sodium triacetoxyborohydride (4.50 g, 21.2 mmol) was added to a stirred solution of **19** (1.50 g, 4.24 mmol) in acetonitrile (150 mL). The reaction mixture was allowed to stir for 20 min at room temperature and then quenched with water (250 mL) and extracted with dichloromethane (3 × 150 mL), dried (Na_2SO_4), and evaporated under reduced pressure. Purification by column chromatography (EtOAc/EtOH, 9:1) followed by recrystallization from ethyl alcohol and diethyl ether yielded compound **20** (1.71 g, 92%) as colorless crystals: mp 153 °C. ^1H NMR (200 MHz, $CDCl_3$) δ 1.78 (br s, 1H), 2.08 (dd, J = 5.2, J = 11.3, 1H), 2.54−2.68 (m, 4H), 3.72−3.81 (m, 4H), 3.98 (dd, J = 2.0, J = 12.5, 1H), 4.12 (br s, 1H), 4.67 (br d, J = 4.7, 1H), 5.33 (dd, J = 5.2, J = 11.3, 1H), 6.28 (d, J = 11.5, 1H), 7.11−7.17 (m, 2H), 7.32−7.43 (m, 3H), 7.62 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.6, 42.6, 43.9, 64.6, 74.4, 76.3, 89.2, 108.0, 126.5, 127.6, 129.4, 144.4, 144.9, 148.5, 149.6, 162.9. EIMS

m/*z* 356 (M + H)$^+$. Anal. Calcd for $C_{18}H_{21}N_5O_3 \cdot 1.5H_2O$: C, 56.53; H, 6.32; N, 18.31. Found: C, 56.72; H, 6.01; N, 18.06.

8-(2′-Deoxy-β-D-ribofuranosyl)-2-methyl-4-(*N*-methylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (21) was prepared from **20** as described for **17a**: colorless solid (73%); mp 156 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.90 (d, J = 3.1, 1H), 2.11 (dd, J = 5.4, J = 11.2, 1H), 2.53−2.65 (m, 4H), 3.23 (d, J = 5.0, 3H), 3.72−3.80 (m, 1H), 3.95−4.00 (dd, J = 1.9, J = 12.5, 1H), 4.13 (br s, 1H), 4.69 (br t, J = 3.9, 1H), 5.40 (dd, J = 5.4, J = 11.2, 1H), 6.28 (dd, J = 11.8, J = 1.9, 1H), 6.56 (br d, J = 4.4, 1H), 7.86 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.8, 27.7, 44.2, 64.5, 74.3, 76.0, 89.1, 109.8, 144.5, 146.3, 149.6, 164.1. EIMS *m*/*z* 280 (M + H)$^+$. Anal. Calcd for $C_{12}H_{17}N_5O_3 \cdot 0.1H_2O$: C, 51.27; H, 6.17; N, 24.91. Found: C, 51.56; H, 6.06; N, 24.79.

8-(2′-Deoxy-β-D-ribofuranosyl)-2-methyl-4-(*N*-methylamino)pyrazolo[1,5-*a*]-1,3,5-triazine-3′,5′-bis(dibenzyl phosphate) (22). Potassium *tert*-butoxide (1.0 M in THF, 5.5 mL) was slowly added, at − 40°C, to a stirred solution of **21** (700 mg, 2.5 mmol) in anhydrous THF (100 mL). After 5 min, tetrabenzyl pyrophosphate (2.59 g, 5.5 mmol) was added and stirring was continued for 15 min at −40 °C. The reaction mixture was allowed warm to 0 °C, then quenched with acetic acid (250 μL). The mixture was diluted with ethyl acetate (200 mL), washed with ice-cold water (100 mL), dried (Na_2SO_4), and concentrated to dryness under reduced pressure. Chromatography on silica (EtOAc/CH_2Cl_2/EtOH, 40:50:10) followed by recrystallization from ethanol and diethyl ether yielded compound **22** (2.9 g, 66%) as colorless crystals: mp 120 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35−2.41 (m, 2H), 2.52 (s, 3H), 3.20 (d, J = 4.9, 3H), 4.08 (t, J = 4.5, 2H), 4.19−4.24 (m, 1H), 4.99−5.12 (m, 9H), 5.39 (t, J = 8.1, 1H), 6.43 (q, J = 5.2, 1H), 7.28−7.37 (m, 20H), 7.90 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.4, 27.6, 39.8, 67.4, 69.8, 72.1, 80.3, 83.7, 107.7, 128.4, 129.1, 136.1, 138.5, 144.3, 147.1, 149.4, 164.2. EIMS *m*/*z* 800 (M + H)$^+$.

8-(2′-Deoxy-β-D-ribofuranosyl)-2-methyl-4-(*N*-methylamino)pyrazolo[1,5-*a*]-1,3,5-triazine-3′,5′-bisphosphate (2). A mixture of **22** (1.2 g, 1.5 mmol) and 10% Pd/C (1.0 g) in absolute methanol (200 mL) was shaken in a hydrogenation apparatus under 60 psi pressure at room temperature for 1.4 h. The catalyst was removed by filtration and washed with water, and the filtrate was concentrated to dryness. Recrystallization from ethanol and diethyl ether yielded compound **2** (600 mg, 91%) as colorless crystals: mp 145 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.26−2.41 (m, 5H, 2′-H), 3.00 (d, J = 4.7, 3H), 3.85−3.98 (m, 2H), 4.10−4.18 (m, 1H), 4.79−4.84 (m, 1H), 5.25 (dd, J = 5.6, J = 10.0, 1H), 8.14 (s, 1H), 8.61 (q, J = 4.7, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.5, 27.9, 41.5, 66.0, 71.5, 77.8, 84.3, 108.3, 144.7, 147.1, 149.6, 163.6; ^{33}P NMR (300 MHz, D_2O, 80°C) δ 0.46, 1.21. Anal. Calcd for $C_{12}H_{19}N_5O_9P_2 \cdot 2\ H_2O$: C, 30.32; H, 4.87; N, 14.74. Found: C, 30.38; H, 4.76; N, 14.61.

8-(2′-Deoxy-β-D-xylofuranosyl)-2-methyl-4-(*N*-methyl-*N*-phenylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (23) was prepared from **19** and K-selectride as described for **20**: colorless solid (78%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.33 (dd, J = 5.6, J = 14.7, 1H), 2.59−2.79 (m, 4H), 3.76 (s, 3H), 4.02−4.14 (m, 2H), 4.23 (dd, J = 1.5, J = 12.9, 1H), 4.57−4.62 (m, 1H), 5.42 (dd, J = 5.9, J = 9.3, 1H), 7.14−7.19 (m, 2H), 7.34−7.43 (m, 3H), 7.80 (s, 1H). EIMS *m*/*z* 356 (M + H)$^+$. Anal. Calcd for $C_{18}H_{21}N_5O_3$: C, 60.83; H, 5.96; N, 19.71. Found: C, 60.93; H, 6.01; N, 19.84.

8-(2′-Deoxy-β-D-xylofuranosyl)-2-methyl-4-(*N*-methylamino)pyrazolo[1,5-*a*]-1,3,5-triazine (24) was prepared from **23** as described for **17a**: colorless solid (67%); mp 145 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.28−2.36 (m, 1H), 2.54 (s, 3H), 2.67−2.73 (m, 1H), 2.80 (d, J = 5.0, 1H), 3.23 (d, J = 5.3, 3H), 3.95−4.08 (m, 3H), 4.40−4.51 (m, 1H), 5.11 (dd, J = 6.8, J = 8.7, 1H), 5.82 (d, J = 10.6, 1H), 6.62 (br s, 1H), 7.87 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.9, 27.7, 43.2, 63.2, 71.4, 74.2, 82.4, 110.4, 144.8, 145.8, 149.6, 164.2. EIMS *m*/*z* 280 (M + H)$^+$. Anal. Calcd for $C_{12}H_{17}N_5O_3$: C, 51.60; H, 6.13; N, 25.07. Found: C, 51.63; H, 6.27; N, 25.11.

8-(2′-Deoxy-β-D-xylofuranosyl)-2-methyl-4-(*N*-methylamino)pyrazolo[1,5-*a*]-1,3,5-triazine-3′,5′-cyclic phosphate benzyl ester (25) was prepared from **24** as described for **22**: colorless solid (63%); mp 182 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (dd, J = 5.3, J = 14.4, 1H), 2.54 (s, 3H), 2.70−2.78 (m, 1H), 3.22 (d, J = 5.0, 3H), 3.82−3.85 (m, 1H), 4.39−4.62 (m, 2H), 4.92−4.94 (m, 1H), 5.16 (dd, J = 0.6, J = 8.1, 2H), 5.46 (dd, J = 5.6, J = 9.4, 1H), 6.54 (q, J = 5.0, 1H), 7.37−7.45 (m, 5H), 8.17 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.4, 27.6, 41.4, 67.9, 69.5, 70.6, 75.1, 81.7, 109.4, 128.6, 129.1, 129.2, 136.0, 144.6, 146.7, 149.4, 164.3. EIMS *m*/*z* 432 (M + H)$^+$. Anal. Calcd for $C_{19}H_{22}N_5O_5P$: C, 52.90; H, 5.14; N, 16.23. Found: C, 53.01; H, 5.15; N, 16.18.

8-(2′-Deoxy-β-D-xylofuranosyl)-2-methyl-4-(*N*-methylamino)pyrazolo[1,5-*a*]-1,3,5-triazine-3′,5′-cyclic phosphate (26) was prepared from **25** as described for **2**: colorless solid (92%); mp 156 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 2.28 (dd, J = 4.1, J = 14.7, 1H), 2.69 (s, 3H), 2.70−2.87 (m, 1H), 3.28 (s, 3H), 3.94−3.97 (m, 1H), 4.41−4.63 (m, 2H), 4.98−5.01 (m, 1H), 5.39 (dd, J = 4.1, J = 9.4, 1H), 8.21 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.4, 28.0, 41.5, 67.6, 70.2, 75.3, 80.8, 110.1, 144.8, 146.4, 149.6, 163.6. EIMS *m*/*z* 342 (M + H)$^+$. Anal. Calcd for $C_{12}H_{16}N_5O_5P$: C, 42.23; H, 4.73; N, 20.52. Found: C, 42.09; H, 5.03; N, 20.48.

Biological Tests: Washed Human Platelet Aggregation. Washed human platelets were prepared as previously described[1] and resuspended at 3 × 10^5 platelets/μL in Tyrode's buffer containing 2 mM $CaCl_2$, in the presence of 0.02 U/mL of the ADP scavenger apyrase (adenosine 5′-triphosphate diphosphorylase, EC 3.6.1.5). Platelets were kept at 37 °C throughout all experiments and aggregation was measured by standard methods.[1]

Briefly, a 450-μL aliquot of platelet suspension was stirred at 1100 rpm and activated by addition of agonists and of human fibrinogen (0.8 mg/mL), in a final volume of 500 μL. The extent of aggregation was estimated quantitatively by measuring the maximum curve height above the baseline.

$[Ca^{2+}]_i$ Measurements. Fura-2/AM loaded human platelets were prepared as previously described[1] and resuspended in Tyrode's buffer with 2 mM $CaCl_2$. Aliquots of fura-2-loaded platelets were transferred to a 10 × 10 mm^2 quartz cuvette maintained at 37 °C and fluorescence measurements were performed under continuous stirring, in a spectrofluorimeter.[1] The excitation wavelength was alternately fixed at 340 or 380 nm, fluorescence emission was determined at 510 nm, and results were calculated as the fluorescence ratio (340/380) in arbitrary units.

In vivo studies. Male Wistar rats weighing 300 g (Iffa-Credo, l'Arbresle, France) were anesthetized by intraperitoneal injection of 200 μL of xylazine base (0.2 mg/kg) and ketamine (1 mg/kg). At time zero, **2** (30 mg/kg) or vehicle was injected into the penis vein. Blood (6.3 mL) was drawn (1 and 30 min) later from the abdominal aorta into syringes containing 0.7 mL of 3.15% sodium citrate and immediately centrifuged (70 s at 1570*g*) at room temperature. Citrated platelet-rich plasma (cPRP) was removed and platelets were adjusted to 5 × 10^5/μL with platelet-poor plasma (PPP). Platelet aggregation was measured in citrated platelet-rich plasma from control and compound **2**-treated rats.

Determination of the pK_a. To solutions of compound **7** at a concentration of ca. 3 × 10^{-3} mol dm^{-3} was added 1.2 equiv of HCl before the solution was titrated with potassium hydroxide. Due to the poor solubility of the compound in aqueous medium, the titration was performed in CH_3OH/H_2O 50:50 at 20 °C in the presence of 0.2 M KCl in order to hold the ionic strength constant. The pH measurements were made

(30) Boyer, J. L.; Mohanram, A.; Gamaioni, E.; Jacobson, K. A.; Harden, T. K. *Br. J. Pharmacol.* **1998**, *124*, 1−3.

(31) Gans, P.; Sabatini, A.; Vacca, A. Investigation of equilibria in solution. Determination of equilibrium constants with the HYPERQUAD suite programs. *Talanta* **1996**, *43*, 1739−1753.

in 2 cm^3 solutions with a combined semimicroglass electrode connected to a pH meter. The electrode was calibrated in terms of concentrations at pH 2 and its slope was checked by a previous acid−base titration. The titration curves allowed the calculation of the total acid (C_H), whereas the concentration of the ligands (C_L) was obtained by weight. Since the pK_a values appeared to be very low, the average degree of protonation $\bar{p}$ versus pH was calculated according to the following equation: $\bar{p} = (C_H - [H^+] + [OH^-])/C_L$, to provide an initial constant for the refinement by HYPERQUAD.[31] Compound **7** remains soluble at a millimolar concentration over all the studied pH range (2.4 to 10.5) in the CH_3OH/H_2O 50:50 medium. The $\bar{p}$ reaches 0.75 and its pK_a value was calculated to be 3.02 ± 0.04. Addition of 1.2 equiv of H^+ to **7** in aqueous medium led to its solubilization by protonating it at 80% according to the $\bar{p} = f$(pH) curve.

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